22:11:33 OCA PAD AMENDMENT - PROJECT HEADER INFORMATION 08/11/91

Active

Project #: E-19-613 Cost share #: E-19-347 Rev #: 8
Center # : R6446-0A0 Center shr #: F6446-0A0 OCA file #:
Work type : RES
Contract#: CTS-8722281 Mod #: ADMIN #3 Document : GRANT
Prime #: Contract entity: GTRC

Subprojects ? : N CFDA: 47.041
Main project #: PE #: N/A

Project unit: CHEM ENGR Unit code: 02.010.114
Project director(s):
ROUSSEAU R W CHEM ENGR (404)894-2867

Sponsor/division names: NATL SCIENCE FOUNDATION / GENERAL
Sponsor/division codes: 107 / 000

Award period: 880101 to 920630 (performance) 920930 (reports)

Sponsor amount	New this change	Total to date
Contract value	0.00	216,816.00
Funded	0.00	216,816.00
Cost sharing amount		2,729.00

Does subcontracting plan apply ?: N

Title: CRYSTAL PURITY, HABIT, AND SIZE DISTRIBUTION FROM BATCH CRYSTALLIZATION

PROJECT ADMINISTRATION DATA

OCA contact: Mildred S. Heyser 894-4820

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Security class (U,C,S,TS) : U ONR resident rep. is ACO (Y/N): N
Defense priority rating : NSF supplemental sheet
Equipment title vests with: Sponsor GIT X

Administrative comments -
AMENDMENT 3 EXTENDS PERFORMANCE PERIOD TO 6/30/92. ALL OTHER TERMS AND CONDITIONS REMAIN UNCHANGED.

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

NOTICE OF PROJECT CLOSEOUT

Closeout Notice Date 02/04/93

Project No. E-19-613 Center No. R6446-0A0

Project Director ROUSSEAU R W School/Lab CHEM ENGR

Sponsor NATL SCIENCE FOUNDATION/GENERAL

Contract/Grant No. CTS-8722281 Contract Entity GTRC

Prime Contract No.

Title CRYSTAL PURITY, HABIT, AND SIZE DISTRIBUTION FROM BATCH CRYSTALLIZATION

Effective Completion Date 920630 (Performance) 920930 (Reports)

Closeout Actions Required:	Y/N	Date Submitted
Final Invoice or Copy of Final Invoice	N	
Final Report of Inventions and/or Subcontracts	N	
Government Property Inventory & Related Certificate	N	
Classified Material Certificate	N	
Release and Assignment	N	
Other	N	

CommentsLETTER OF CREDIT APPLIES.

Subproject Under Main Project No.

Continues Project No.

Distribution Required:

Project Director	Y
Administrative Network Representative	Y
GTRI Accounting/Grants and Contracts	Y
Procurement/Supply Services	Y
Research Property Managment	Y
Research Security Services	N
Reports Coordinator (OCA)	Y
GTRC	Y
Project File	Y
Other HARRY VANN-FMD	Y
FRED CAIN-OOD	Y

E-19-613

—A Progess Report on CBT-8722281—

Crystal Purity, Habit, and Size Distribution from Batch Crystallization

to

National Science Foundation
Separation and Purification Processes
1800 G Street, N.W.
Washington, D. C. 20550

by

Ronald W. Rousseau
School of Chemical Engineering
Georgia Institute of Technology
Atlanta, Georgia 30332-0100

February 1989

Introduction

This report provides a summary of progress to date on NSF Grant Number CBT-8722281, "Crystal Purity, Habit, and Size Distribution from Batch Crystallization." The effective award date was January 1, 1988 but the project was not established until February 8, 1988.

Among the objectives of the research supported by this grant is elucidation of the factors that determine the purity of crystals obtained in the separation and purification of high-value chemicals. The initial model compounds selected for study are the commercially important amino acids L-isoleucine, L-leucine, and L-valine. The research focuses on batch crystallization because most of the processing utilized in specialty-chemical production involves such units and because most earlier research was on continuous crystallizers used in the production of commodity chemicals. Processing improvements resulting from this research will contribute to enhanced product quality and process reliability.

Personnel

Mr. Timothy Gambrel, a graduate student in the School of Chemical Engineering, joined the research group at the beginning of the project and has been responsible for much of the experimental work described below. Ms. Cynthia Moody, a chemical engineering undergraduate, assisted in the laboratory during Summer Quarter 1988. Ms. Susan Caudle and Mr. Herve Charmolue have joined the research group effective Winter Quarter 1989.

Experimental Equipment

A major portion of the research project requires determination of the effects of process variables on the purity of recovered crystals. Three experimental batch crystallizers have been constructed. These are well-stirred vessels made of glass and jacketed for temperature control. Each is to be operated so as to generate supersaturation in a mode different from the other two units; cooling, acid addition, and evaporation are the modes of generating supersaturation that are to be studied. Most of the research performed to date has utilized cooling to provide the driving force for crystallization.

The objectives of the research required solubility data on the systems of interest and an accurate analysis of the composition of recovered crystals and mother liquor. Procedures have been developed for these measurements:

- Solubility. Determination of solubility data requires measurement of the concentration of the crystallizing species in a solution that is in equilibrium with crystals. A number of procedures could be used for such measurements, but most are lengthy or of insufficient precision. Using funds from the grant, a densitometer was purchased and set up for performing such measurements. An example calibration curve of density versus L-isoleucine concentration which was obtained with this instrument is shown in Figure 1. Precision of the instrument is to the sixth decimal place, but such accuracy requires that the temperature of the sample be maintained constant to $\pm 0.01\,^{\circ}C$.
- Compositional Analysis. The effects of a number of process variables on crystal purity are to be determined. Compositional analysis of solutions and crystals is to be determined by utilizing high-performance liquid chromatography (HPLC) using a pre-column OPA derivatization method.

Summary of Experimental Observations

As indicated earlier, the results to date have been obtained by study of a model system consisting of three amino acids: L-isoleucine, L-leucine, and L-valine.

Solubility of L-Isoleucine

The solubility of L-isoleucine depends on pH and, in the present work, solubilities were determined near the isoelectric point. These data are to be used in the analysis of the crystallization of L-isoleucine in neutral form. (At low pH, L-isoleucine crystallizes in an acid form.) Figure 2 shows experimentally determined solubilities using two methods for bringing the two-phase system to equilibrium. In one, a slurry consisting of an excess quantity of L-isoleucine crystals is heated to a specified temperature and maintained at this temperature for 18–20 hours. In the second procedure, the slurry was heated first to a temperature 10 to 20 °C above the desired equilibrium value; it was then allowed to cool to the desired temperature where it was maintained for 18–20 hours. Solubilities were similar regardless of the procedure for achieving equilibrium.

Purity of L-Isoleucine Crystals

L-valine and L-leucine are common impurities found in the fermentation broth from which L-isoleucine is recovered. Several series of experiments were performed to investigate the purity of L-isoleucine when it was crystallized from a neutral solution containing L-valine and

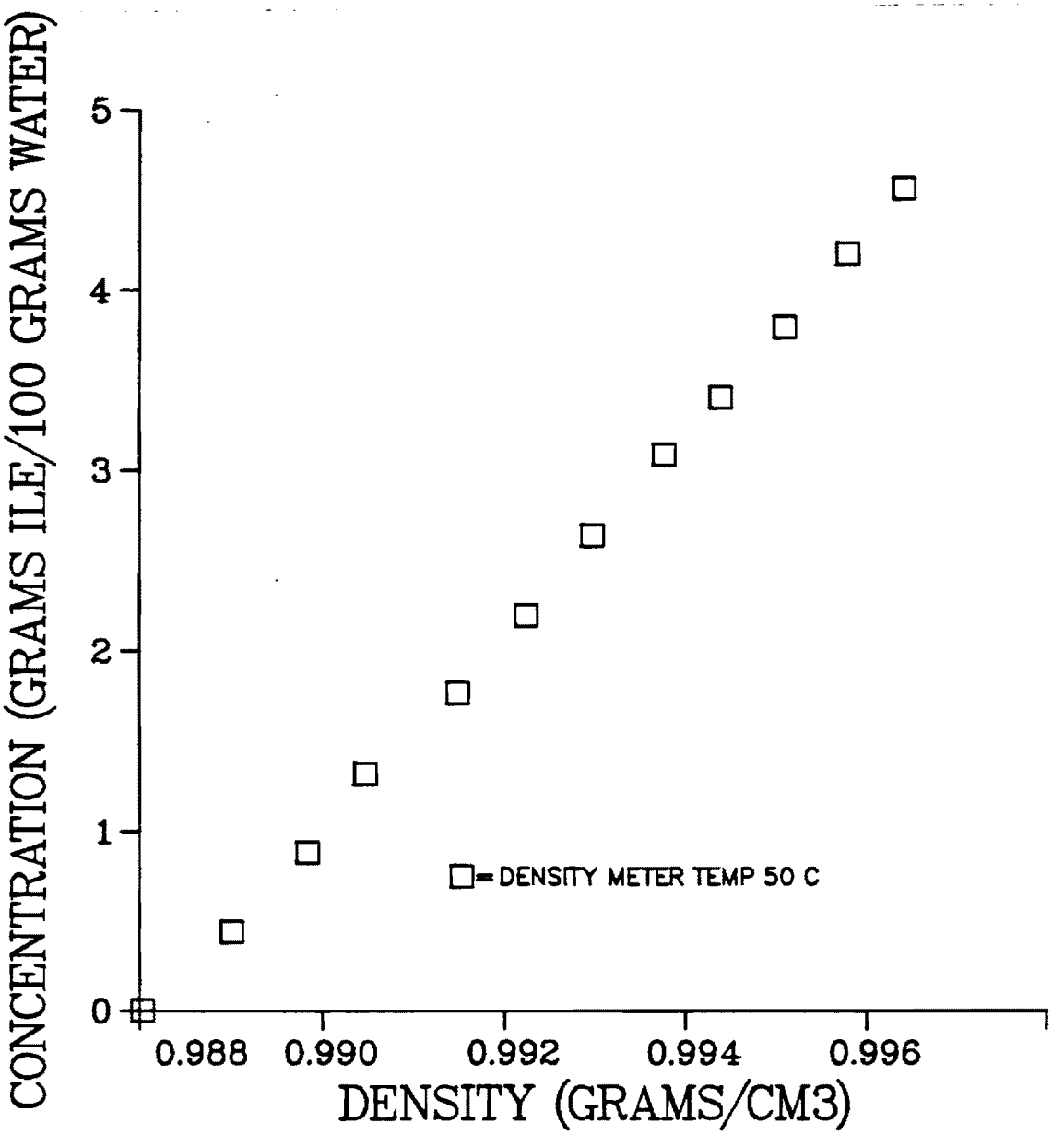

Figure 1: Calibration Curve: Solution Density vs. Concentration of L-Isoleucine at °C

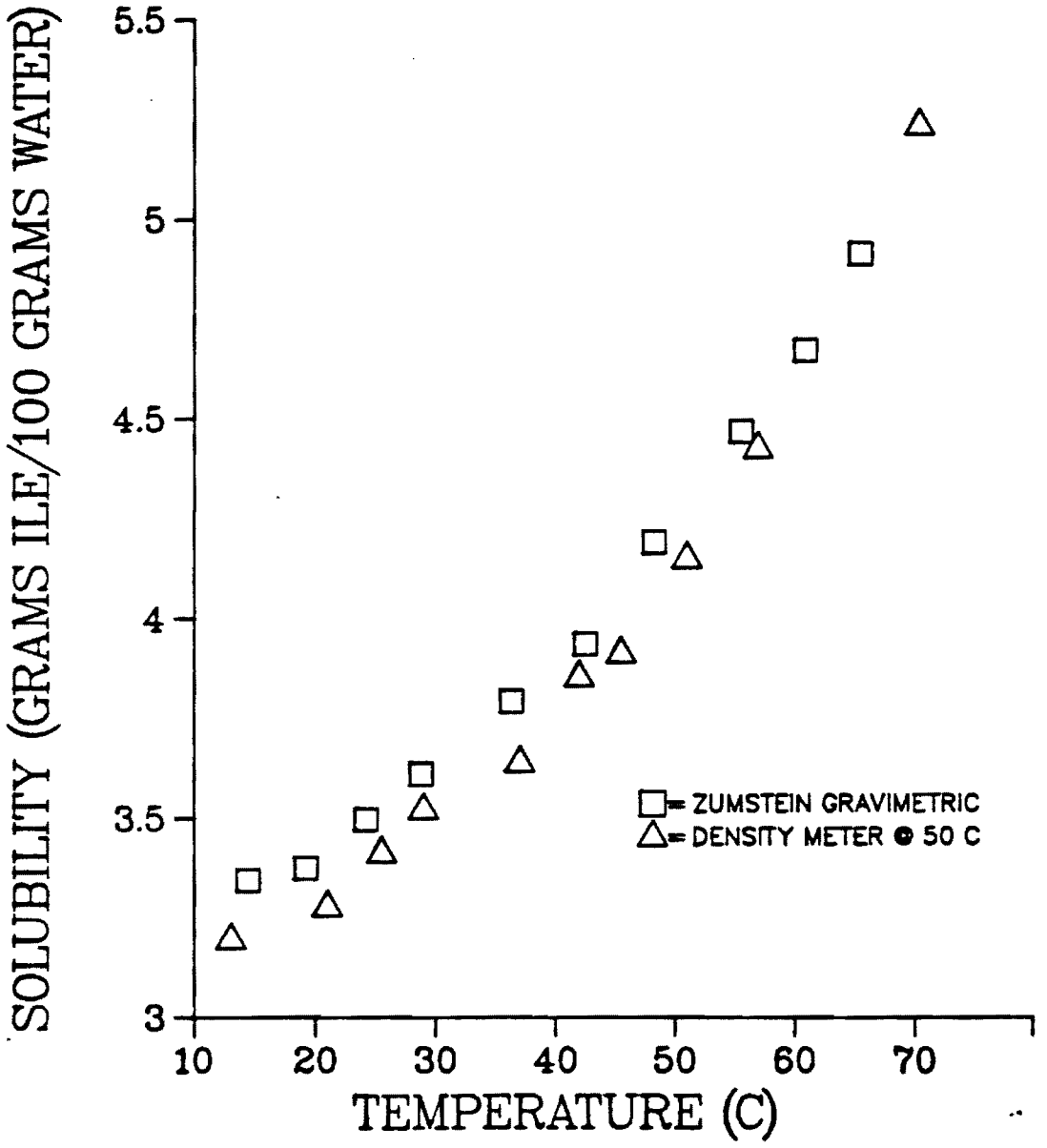

Figure 2: Solubility of Neutral L-Isoleucine

L-leucine. Some of the experimental variables investigated early in the study include initial concentration of the impurity in the solution, crystal washing, and agitation in the crystallizer. For the purposes of illustrating the nature of the experimental work being done under this grant, only a summary of the results examining initial solution concentration will be described.

In following the discussion it is helpful to use a term that reflects the tendency of an impurity to partition between recovered crystals and residual solution. A partition coefficient, P_i, is defined here for that purpose as follows:

$$P_i = \frac{R_{i,cry}}{R_{i,sol}} \tag{1}$$

where $R_{i,cry}$ is the ratio of moles of impurity i to moles of L-isoleucine in the recovered crystal and $R_{i,sol}$ is the ratio of moles of impurity i to moles of L-isoleucine in the solution from which L-isoleucine is crystallized.

A series of experiments were performed in which all conditions and procedures were maintained constant from experiment to experiment except for the concentrations of the impurities L-leucine and L-valine. Recovered L-isoleucine crystals were washed and analyzed with the results shown in Figures 3 and 4. These figures show several important features. First, it is apparent that the processing conditions had little detectable influence on the partitioning of L-valine; either the concentration of valine had no effect on the amount of valine in the recovered crystals or the detection limits of the analytical procedures were exceeded by extremely low concentrations of valine in the crystals. Second, L-leucine partitions differently depending on the concentration in solution. As expected, the amount of L-leucine found in the recovered crystals increased with an increase in solution concentration over both composition ranges covered by Figures 3 and 4. Importantly for the process of purification, P_{leu} is less than 1 for the composition range covered by Figure 3. This means that leucine is preferentially excluded from the isoleucine crystals. Figure 4 shows quite different behavior, however; the partition coefficient actually becomes greater than 1 as L-leucine concentration in solution becomes less than about 0.0025 mol L-leucine per mol of L-isoleucine. These results show that L-leucine would prefer be in the crystalline phase when the solution concentration is low. The implications of these results raise significant concerns regarding the suitability of simple crystallization as a means for purifying L-isoleucine from solutions containing trace quantities of impurities.

Budgetary Status

Expenditures for the first year of this project have matched with the estimated budget except in the area of graduate student support. Only one student was supported during the first year, whereas the budget had funds for two. It is hoped that these funds can be carried

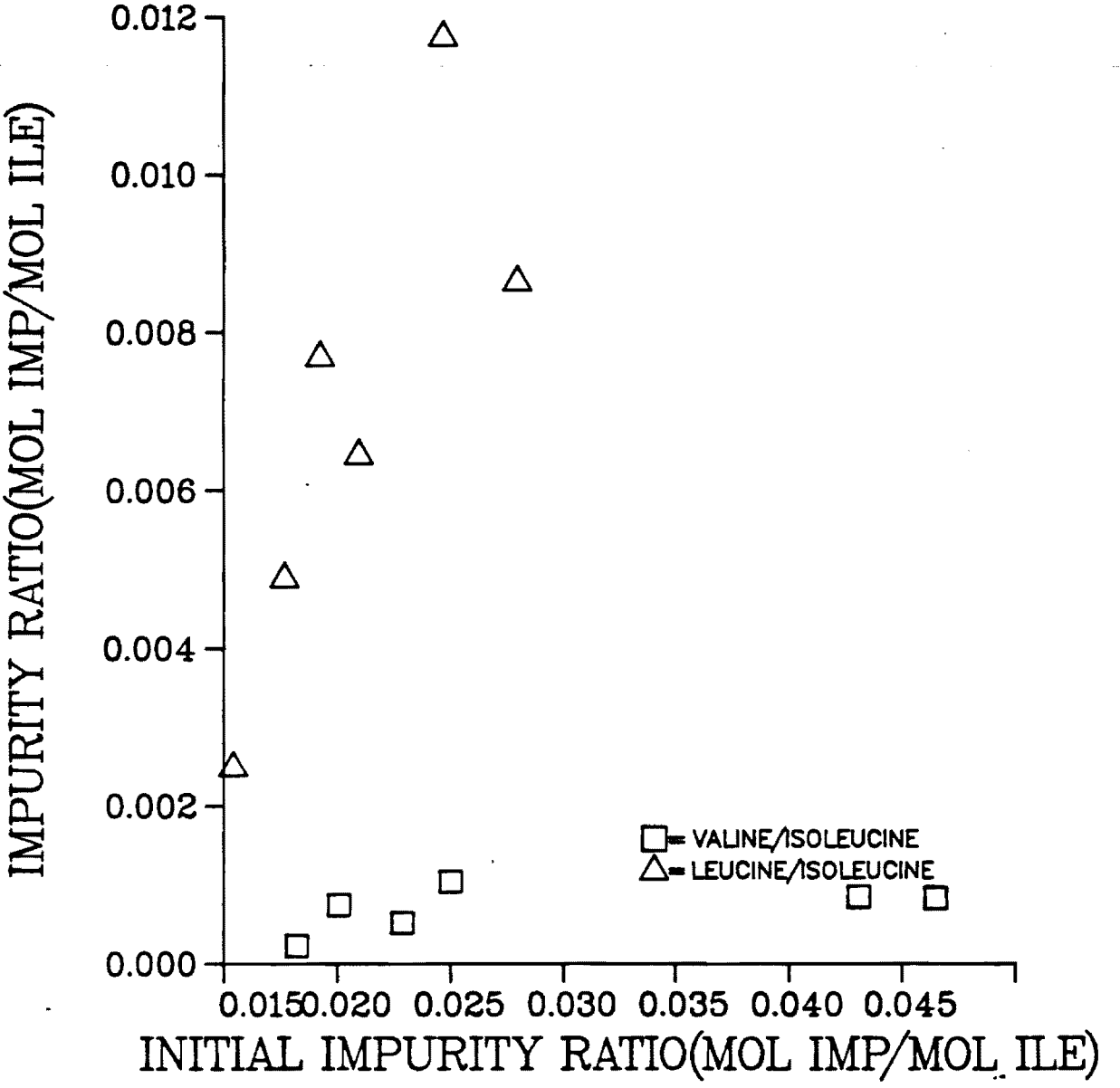

Figure 3: Effects of Initial Concentrations of L-Leucine and L-Valine on the Purity of L-Isoleucine Crystals (High Impurity Concentrations)

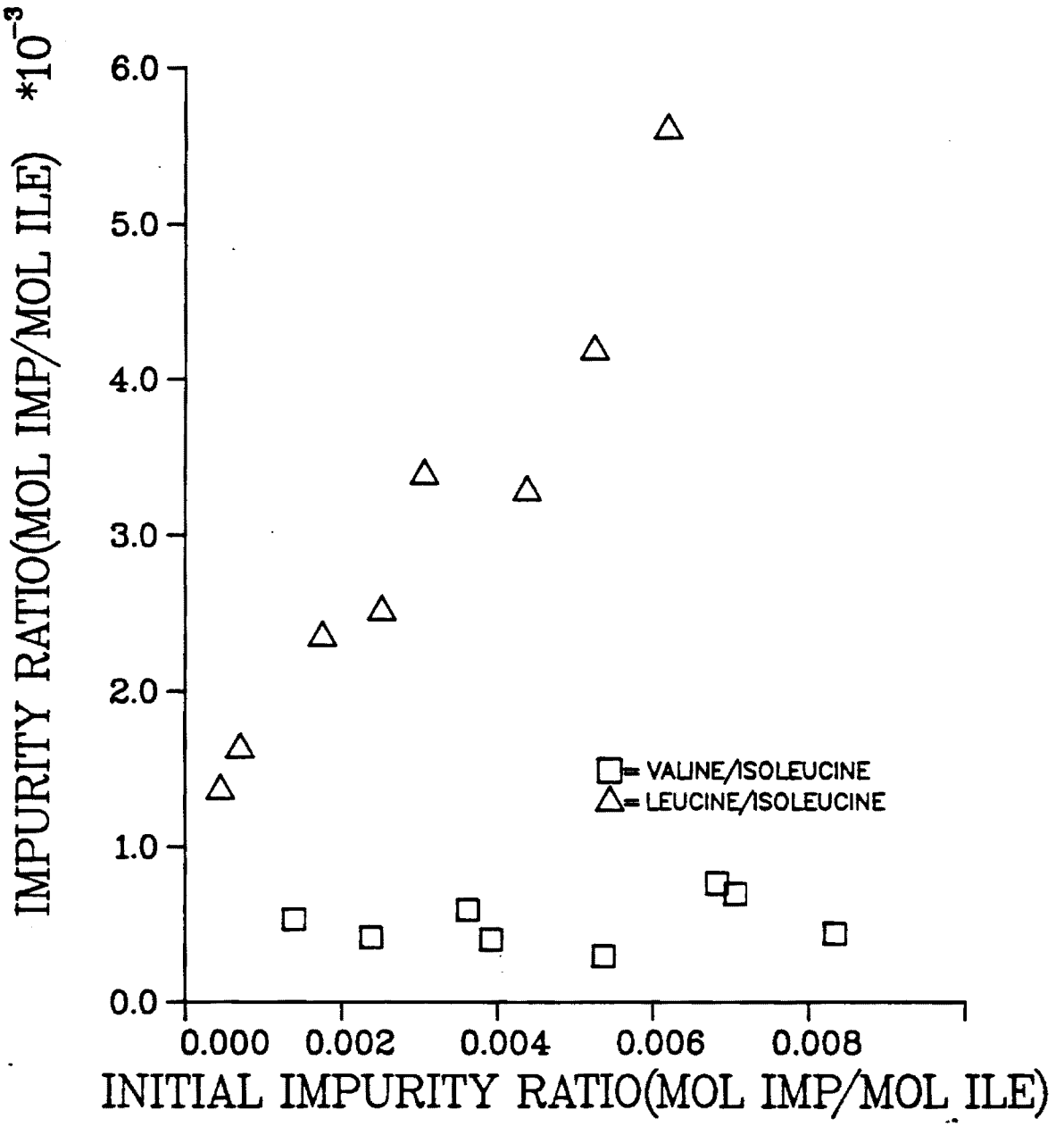

Figure 4: Effects of Initial Concentrations of L-Leucine and L-Valine on the Purity of L-Isoleucine Crystals (Low Impurity Concentrations)

forward into the second year so that support for three students will be available. This will allow completion of the work by Mr. Timothy Gambrel, continued support for Mr. Herve Charmolue, and the addition of two new graduate students to the research effort. Accordingly, full funding in the amount of $67,050 is requested for the second year of this grant.

E-19-613

—A Progess Report on CBT-8722281—

Crystal Purity, Habit, and Size Distribution from Batch Crystallization

to

National Science Foundation
Interfacial, Transport, and Separation Processes Program
Division of Chemical and Thermal Systems
1800 G Street, N.W.
Washington, D. C. 20550

by

Ronald W. Rousseau
School of Chemical Engineering
Georgia Institute of Technology
Atlanta, Georgia 30332-0100

January 1990

Contents

Introduction

This report provides a summary of progress to date on NSF Grant Number CBT-8722281, "Crystal Purity, Habit, and Size Distribution from Batch Crystallization." Among the objectives of the research supported by this grant is elucidation of the factors that determine the purity of crystals obtained in the separation and purification of high-value chemicals. The initial model compounds selected for study are the commercially important amino acids L-isoleucine, L-leucine, and L-valine. Additionally, research has been initiated on L-serine, specifically focused on solvent uptake during crystallization.

The research examines batch crystallization because most of the processing utilized in specialty-chemical production involves such units and because most earlier research was on continuous crystallizers used in the production of commodity chemicals. Processing improvements resulting from this research will contribute to enhanced product quality and process reliability.

Personnel

Mr. Timothy Gambrel, a graduate student in the School of Chemical Engineering, joined the research group at the beginning of the project and has been responsible for much of the experimental work involving L-isoleucine. Mr. Gambrel received his M.S. degree in June 1989 and is now employed by The Dow Chemical Company. Ms. Cynthia Moody, a chemical engineering undergraduate, assisted in the laboratory during Summer Quarter 1988. The Ph.D. research of Mr. Paul Wang was to focus on the use of supercritical solvents to obtain highly pure crystalline materials. Although he spent much of 1989 constructing an apparatus and carrying out preliminary experiments, personal (nonacademic) reasons have forced Mr. Wang to forego completion of his work. Mr. Herve Charmolue joined the research group Winter Quarter 1989 and has been working with L-serine crystallization. Ms. Marena Gatewood is a new graduate student who will join the research group effective Winter Quarter 1990. Dr. Clifford Tai, a chemical engineering faculty member from National Taiwan University, was a visiting scientist at Georgia Tech during part of 1989 and contributed to the research effort in crystallization.

Experimental Equipment

A major portion of the research project requires determination of the effects of process variables on the purity of recovered crystals. Three experimental batch crystallizers have been constructed. These are well-stirred vessels made of glass and jacketed for temperature control. Each is to be operated so as to generate supersaturation in a mode different from the other two units; cooling, acid or nonsolvent addition, and evaporation are the modes of generating supersaturation that are to be studied. Most of the research on L-isoleucine utilized cooling or acid addition to provide the driving force for crystallization, while the work on L-serine examined the addition of a nonsolvent, methanol.

A second area of investigation that was opened during the past year is the utilization of supercritical solvents to produce highly pure, tailored crystalline products. A high-pressure apparatus was constructed and several preliminary experiments conducted.

Summary of Research Accomplishments

As indicated earlier, the results to date have been obtained by study of model systems consisting of amino acids.

Solubility Studies

The solubility of L-isoleucine depends on pH and temperature. A manuscript (R. C. Zumstein and R. W. Rousseau, "Solubility of L- Isoleucine in and Recovery of L-Isoleucine from Neutral and Acidic Aqueous Solutions," *Industrial & Engineering Chemistry Research*, **28**, 1226–1231(1989)) which is based partially on work performed as part of the present study is attached. Solubilities of L-serine in aqueous ethanol solutions have also been determined and are reported in the attached progress report from Mr. Charmolue.

Purity of L-Isoleucine Crystals

L-valine and L-leucine are common impurities found in the fermentation broth from which L-isoleucine is recovered. Several series of experiments were performed to investigate the purity of L-isoleucine when it was crystallized from a neutral solution containing L-valine

and L-leucine. Some of the experimental variables investigated include initial concentration of the impurity in the solution, crystal washing, and agitation in the crystallizer.

In following the discussion it is helpful to use a term that reflects the tendency of an impurity to partition between recovered crystals and residual solution. A partition coefficient, P_i, is defined here for that purpose as follows:

$$P_i = \frac{R_{i,cry}}{R_{i,sol}} \tag{1}$$

where $R_{i,cry}$ is the ratio of moles of impurity i to moles of L-isoleucine in the recovered crystal and $R_{i,sol}$ is the ratio of moles of impurity i to moles of L-isoleucine in the solution from which L-isoleucine is crystallized.

Experiments have shown that P_i can be considered a constant except at values of $R_{i,sol}$ between 0.01 and 0.08 mol/mol of L-isoleucine. At $R_{i,sol}$ in the range of 0.001 to 0.006 P_i is constant but different from the value at the higher solution concentrations of impurities. In fact, in the lower range of $R_{i,sol}$, P_i was observed to be greater than one.

Several presentations, either partially or completely based on research from the present work, have been given at professional society meetings, universities, and industrial research laboratories. These are listed below:

- "Use of Crystallization for Recovery and Purification of L-Isoleucine and Other Biologically Produced Materials," Department of Chemical Engineering, Louisiana State University, Baton Rouge, LA, March 1988.
- "Crystallization for Separation and Purification," Dow Chemical Company, Midland, MI, March 1988.
- "Relating Crystal Properties to Crystallizer Operation," Ajinomoto USA, Raleigh, NC, September 1988.
- "Removal of Trace Impurities by Crystallization," AIChE Annual Meeting, Washington, DC, November 1988.
- "Crystallization Processes for Recovery and Purification of High-Value Chemicals," Department of Chemical Engineering, Auburn University, Auburn, AL, January 1989.
- "Crystallization Processes for Recovery and Purification of High-Value Chemicals," Department of Chemical Engineering, University of New Mexico, Albuquerque, NM, January 1989.

- "Crystallization Processes for Recovery and Purification of High-Value Chemicals," Department of Chemical Engineering, Texas A&M University, College Station, TX, February 1989.
- "Use of Crystallization for Separation and Purification, Or.....What's New in Crystallization," Separations Symposium, E. I. DuPont, Wilmington, DE, April 1989.
- "Separation and Purification of Amino Acids by Crystallization," Engineering Foundation Conferences, Davos, Switzerland, May 1989.
- "Principles of Batch Crystallization," General Electric Specialty Chemicals, Morgantown, WV, July 1989.
- "Yield and Purity of L-Isoleucine Recovered by Crystallization," Ajinomoto Co., Inc., Technology and Engineering Center, Central Research Laboratories, Kawasaki, Japan, August 1989.
- "Separation and Purification by Crystallization," Department of Chemical Engineering, Purdue University, West Lafayette, IN, October 1989.
- "The Effects of Crystallizer Operating Variables on Crystal Purity," AIChE Annual Meeting, San Francisco, CA, November 1989.
- "Purification of Amino Acids by Batch Crystallization," The 1989 International Chemical Congress of Pacific Basin Societies (PACIFICHEM '89), Honolulu, HI, December 1989.

A manuscript based on the research will be submitted for publication shortly.

Budgetary Status

Expenditures for the first two years of this project have matched with the estimated budget except in the area of graduate student support. It is recommended that these funds can be carried forward so that support for three students will be available. Accordingly, full funding in the amount of $67,396 is requested for the third year of this grant.

From the experimental data, it was found that the adsorptive capacities of the regenerated activated carbon for benzene and toluene after many cycles were still close to those of the virgin carbon and remained stable. The effects of temperature, pressure, and flow rate on regeneration efficiency were also studied. At higher pressures the regeneration was found to be more favorable. But as the temperature effect is concerned, an optimal temperature was observed when the pressure was above 100 atm. Because of the regeneration efficiency varied with the flow rate, the interphase mass-transfer resistance may play an important role under supercritical operations. A mathematic model, assuming the regeneration rate depended on both the benzene and toluene concentrations on activated carbon, was proposed in this study, which was found to agree well with the experimental data.

The adsorption rates of benzene and toluene on the activated carbon regenerated by the supercritical fluid method and the steam method using saturated and superheated steams were also compared in this study. It was observed that the supercritical fluid method offered a better regeneration efficiency than the steam method.

Acknowledgment

Financial support from the National Science Council of ROC and Asia Chemical Corporation in ROC is gratefully acknowledged.

Nomenclature

C_B, C_T = concentration of benzene and toluene, respectively, mol/cm^3
k_B, k_T = desorption rate constants, cm^3/(s·mol)
S_B, S_T = loaded benzene and toluene on activated carbon, respectively, mol/cm^3
$S_{B,0}$, $S_{T,0}$ = initially loaded benzene and toluene on activated carbon, respectively, mol/cm^3
T = temperature, K
t = time, s
z = axial position in the column, cm

Greek Symbols

ϵ = void fraction in the packed column
μ = viscosity, g/(cm·s)
ρ = density, g/cm^3

Registry No. C, 7440-44-0; CO_2, 124-38-9; benzene, 71-43-2; toluene, 108-88-3.

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Tan, C. S.; Liou, D. C. *Sep. Sci. Technol.* 1989, *24*, 111.

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Solubility of L-Isoleucine in and Recovery of L-Isoleucine from Neutral and Acidic Aqueous Solutions

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Recovery and purification of L-isoleucine from fermentation media include several crystallization–recrystallization steps. Solubilities, which were found to be different from those reported by earlier workers, are therefore important in designing these steps and in analyzing crystallizer performance. The effect of temperature on the solubility of L-Ile at the isoelectric point was determined, as was the influence of pH as adjusted by the addition of HCl. Increasing the acid content until there was approximately 1 mol of HCl/mol of L-Ile raised the solubility of L-Ile to a maximum value. Subsequent addition of chloride ions, whether added with more HCl or with inorganic salts, decreased the solubility.

Solubility data for many amino acids in aqueous solutions consist of measurements taken when isolation and detection methods were crude (Greenstein and Winitz, 1961). Isolation was difficult because amino acid preparation involved either chemical synthesis, which resulted in racemic mixtures, or protein hydrolysis, which often resulted in a mixture of several amino acids. Complete resolution of racemic mixtures was difficult, as was the separation of a specific amino acid from a mixture of several other amino acids. The determination of purity was based partially on optical rotation measurements, which could be indecisive in this respect. More recently, biosynthesis methods have been found that produce isomerically pure compounds (Meister, 1965). Also, chromatographic techniques have been developed that give an accurate determination of amino acid purity (Pfeifer et al., 1983).

L-Isoleucine (L-Ile) is an example of the above situation. It is one of the essential amino acids that has been commercially produced by fermentation (Shimura, 1972).

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Crystallization of the neutral form, L-Ile, and the hydrochloric acid salt L-Ile·HCl·H_2O are important in the isolation and recovery from fermentation broths. The motivation for the present work came from the need to know solubilities in order to study methods for improving the yield of product from the crystallization processes and to have an accurate measure of supersaturation, the thermodynamic driving force for crystallization. The solubility of L-Ile in water had been reported by Dalton and Schmidt (1935). Their data were checked, and then the solubility of L-Ile in the presence of hydrochloric acid (HCl) was investigated. In addition, the recovery of L-Ile by precipitatin of L-Ile·HCl·H_2O was examined using various sources of chloride ion as precipitants.

Experimental Section

Analysis of the L-Ile used in the present experiments gave an L-Ile assay of 99.8% with 0.18% other amino acids. The specific rotation, $[\alpha]_D^{25}$, of a dried sample in 6 N HCl was +40.8°. This material was used without any further purification. Deionized water was used in all experiments. ACS-grade sodium chloride (NaCl), potassium chloride (KCl), and ammonium chloride (NH_4Cl) and 37 wt % HCl were used for all preparations.

The solubility data for L-Ile in water were obtained over the temperature range 15–70 °C by agitating slurries of deionized water and an excess of L-Ile crystals. These slurries were heated 10–20 °C above the desired equilibrium temperature, cooled to the desired temperature, and allowed to equilibrate. After 24 h, liquor samples were recovered by filtering the slurries through 0.45-μm filter paper, and the L-Ile contents of these were determined. Several tests were conducted to ensure that 24 h was enough time for the suspensions to come to equilibrium. These tests showed that there was no measurable change in concentration of the samples after 18 h of agitation at a constant temperature.

Solubilities were also obtained for HCl concentrations up to 30 wt % at 25.0 ± 0.1 °C. The experimental procedure entailed adding various amounts of 37 wt % HCl to slurries of L-Ile in deionized water. Mixtures were heated to 60 °C and agitated until all L-Ile was dissolved. The solution was then cooled to 25.0 °C and allowed to equilibrate. If no crystals were present, more L-Ile was added, and the dissolution and cooling cycle were repeated. After 24 h, liquor was recovered by filtration of the slurries through 0.45-μm filter paper and analyzed for L-Ile.

The effect of several chloride ion salts on the solubility of L-Ile in an aqueous HCl solution was also examined at 25.0 ± 0.1 °C. In these experiments, various known amounts of NaCl, KCl, or NH_4Cl were added to a weighed amount of an aqueous solution of 30 wt % L-Ile and 8.5 wt % HCl. The contents were heated to 80 °C and allowed to dissolve completely. The mixture was then slowly cooled to 25.0 °C and allowed to equilibrate. Liquor and crystal samples of the equilibrated slurries were recovered for analysis.

All experiments were conducted in a 600-mL jacketed glass beaker. The temperature was controlled within ±0.05 °C by circulating water from a constant-temperature bath through the jacket of the beaker. The beaker was equipped with three Plexiglas baffles to aid agitation and a cover to minimize evaporation of solvent. A mechanical stirrer provided vigorous agitation. Liquor samples were obtained by filtering the equilibrated slurries through 0.45-μm glass-fiber filter papers. The recovered crystals were washed with acetone and allowed to dry. pH measurements were made with a Ag/AgCl combination electrode. Liquor densities were determined by weighing pipetted liquor volumes of 100.00 ± 0.05 mL. L-Ile concentrations were determined by gravimetric analysis when HCl was absent from the suspension. High-performance liquid chromatography (HPLC) was used to determine L-Ile content when HCl or salts had been added to the slurry. An automated precolumn derivatization technique with o-phthaladehyde (OPA), as outlined by Pfeifer et al. (1983), was utilized for the HPLC. Details of this procedure are given by Zumstein (1987). HCl concentrations were determined by conventional acid-base titration (1987). Salt concentrations were based on known weights of components assuming that all added salt was contained in the liquor. However, NH_4Cl concentrations were checked with a Kjeldahl method (Skoog and West, 1980).

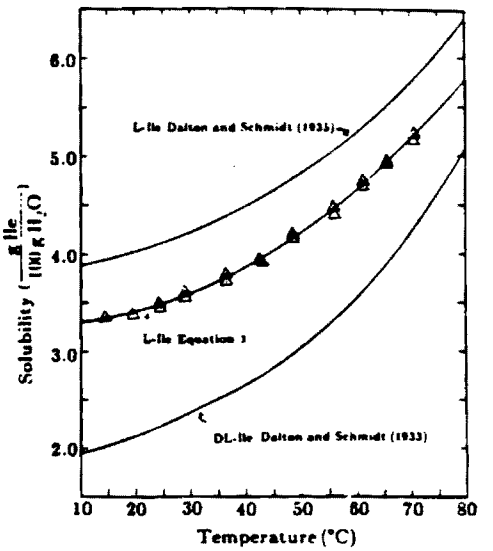

Figure 1. Solubility of L-isoleucine in water.

Results and Discussion

Solubility of L-Ile in H_2O. The solubility data of L-Ile in water determined in the present work are shown in Figure 1, along with the data reported by Dalton and Schmidt (1935). All data have been tabulated by Zumstein (1987). Solubilities are reported in terms of grams of Ile/100 g of H_2O. Note that the solubilities given by Dalton and Schmidt are as much as 15% greater than those reported in this work. One possible reason for this significant deviation could be the purity of the L-Ile. Dalton and Schmidt reported a value of +36.3° for the optical activity, $[\alpha]^{25}_D$, of L-Ile in 6 N HCl in their work; on the other hand, a value of +40.8° was found for L-Ile used in the present work. Greenstein and Winitz (1961) report that the value for the optical activity of L-Ile in 6 N HCl is +40°. Dalton and Schmidt gave no other indication of the purity or source of material used in their work. It is believed that there were impurities in the material used by Dalton and Schmidt, causing the low-value optical activity and the higher solubilities they reported.

Stereoisomers of isoleucine or other amino acids are the most likely impurities in the material used by Dalton and Schmidt. Because isoleucine has two optically active centers, four stereoisomers exist: L, D, L-allo, and D-allo. The L and L-allo as well as D and D-allo forms are referred to as diastereomers and typically have different physical properties. Mixtures of stereoisomers have been found to have solubilities that are different from those of the isomerically pure materials. The effect of the diastereomers on the solubility of L-Ile is uncertain, but Dalton and Schmidt (1933) reported a much lower solubility for a

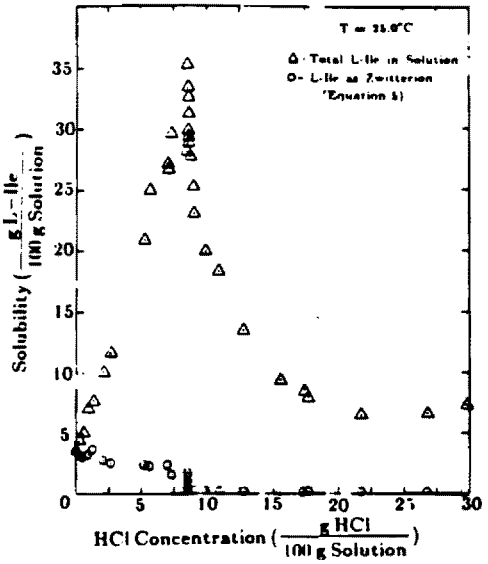

Figure 2. Solubility of L-isoleucine in aqueous HCl solutions.

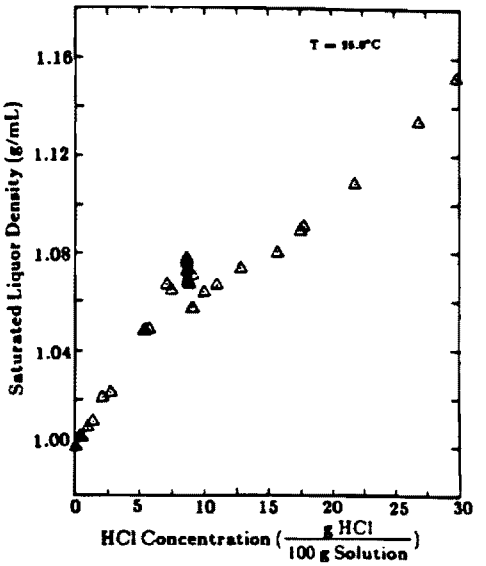

Figure 3. Density of saturated aqueous L-isoleucine solutions in the presence of HCl.

racemic mixture of D and L forms of isoleucine. These data are also shown in Figure 1. Dunn et al. (1933) confirmed these solubilities for the D–L mixture.

Although the presence of D-Ile as the only impurity cannot explain the higher solubilities of L-Ile reported by Dalton and Schmidt (1935), it is an interesting observation. It is typical for a mixture of stereoisomers to exhibit a different solubility from either of the single isomeric forms, even though D and L isomers have identical physical properties. This has been related to forces holding the molecules together in the solid state (Greenstein and Winitz, 1961). On crystallization from racemic mixtures, crystals different from those of the single-isomer crystals are produced. The racemic crystal has a higher crystal density than the single-isomer crystals and has been associated with the lower aqueous solubility. For isoleucine, the density of the D–L crystal is 1.24 (Donnay and Ondik, 1972), while that for the L form has been reported to be 1.20 by Torii and Iitaka (1971). Therefore, isomers of isoleucine adhere to the stated general relationship between crystal density and aqueous solubility.

The dependence of L-Ile solubility on temperature may be expressed by the equation

$$C_s = a_1 + a_2T + a_3T^2 \qquad (1)$$

where C_s is the solubility in grams of the L-Ile/100 g of H_2O and T is temperature in °C. Regression of the data to eq 1 gave values for the parameters a_1, a_2, and a_3 of 3.30 ± 0.04, −0.003 20 ± 0.002 16, and 0.000 430 ± 0.000 025, respectively. The correlation coefficient for the regression was 0.998, and the fit of eq 1 to the data is shown in Figure 1.

Effect of HCl on Solubility. The solubility of L-Ile in the presence of up to 30 wt % HCl at 25.0 °C is shown in Figure 2. The data are also tabulated by Zumstein (1987). The most striking characteristic is that the solubility reaches a maximum at about 8.5 wt % HCl and then sharply decreases as HCl concentration is increased. As shown in Figure 3, the relationship between the density of the saturated liquor and the HCl content also exhibits a sharp change in the region of the solubility peak. In order to explain this behavior, it must be recognized that amino acids can coexist within a solution as acid, base, or zwitterion forms. Each of these species contributes to the total solubility of an amino acid. Only at the isoelectric point is the concentration of acidic and basic forms equal, and there is no net charge on the amino acid.

The solubility of the zwitterion or neutral species is based on an equilibrium with the zwitterion solid,

$$(N^+H_3RCOO^-)_s \rightleftharpoons N^+H_3RCOO^- \qquad (2)$$

and $K_z = [N^+H_3RCOO^-]/[N^+H_3RCOO^-]_s$ is the equilibrium solubility parameter that is dependent on temperature, solvent, and solute concentration. R represents the hydrocarbon chain that defines a specific amino acid. When acid (H^+) is added to the solution at a pH equal to or less than the isoelectric point, the following equilibrium is established between the neutral and acidic forms

$$H^+ + N^+H_3RCOO^- \rightleftharpoons N^+H_3RCOOH \qquad (3)$$

where the equilibrium constant K_a for the reaction is defined as

$$K_a = \frac{[N^+H_3RCOO^-][H^+]}{[N^+H_3RCOOH]} \qquad (4)$$

$[N^+H_3RCOO^-]$ and $[N^+H_3RCOOH]$ represent the solution concentrations of neutral and acidic forms of an amino acid, respectively, while $[H^+]$ represents the free proton concentration. Equation 4 is a constraint on the relative concentrations of the two species in solution. A more common version of this relationship is the Henderson–Hasselbalch equation (Armstrong, 1983):

$$pH = pK_a + \log ([N^+H_3RCOO^-]/[N^+H_3RCOOH]) \qquad (5)$$

where pK_a is the apparent ionization constant for the acid form (2.26 for L-Ile). As acid is added to the solution, the pH of the solution is lowered and the amount of acidic form relative to neutral form is increased. Utilizing eq 5 and the measured values of the solubility (combined acidic and neutral concentrations) and solution pH, the magnitudes of the neutral and acidic forms were determined for acidic L-Ile solutions. Acid has little effect on the neutral species at low acid concentrations, as shown in Figure 2. The total solubility of the amino acid is increased simply due to the increased presence of the acid form of the amino acid.

As the acid content of the solution increases, greater concentrations of the acid form of the amino acid are present in the solution. However, there is a limit to the

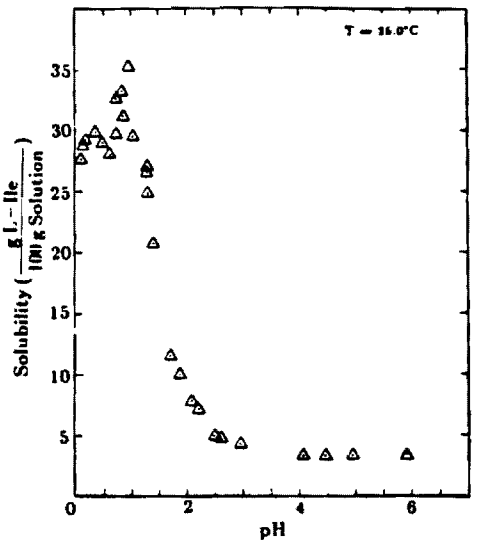

Figure 4. Solubility of L-isoleucine in HCl solutions as a function of pH.

solubility of this species. When HCl is added as the acid, the solubility of the acid form can be related to a equilibrium established with the hydrochloric acid salt,

$$(Cl^-\cdot N^+H_3RCOOH\cdot H_2O)_s \rightleftharpoons N^+H_3RCOOH + Cl^- + H_2O \quad (6)$$

where $Cl^-\cdot N^+H_3RCOOH\cdot H_2O$ is the equilibrium solid phase and the two ions on the right-hand side of the equation are present in the aqueous solution. The solubility product K_{sp} is related to the concentration of the two ions in solution:

$$K_{sp} = [N^+H_3RCOOH][Cl^-] \quad (7)$$

The addition of HCl to an amino acid slurry causes an increase in the acid species concentration simply due to a shift in the equilibrium given by eq 3. It also increases the chloride ion concentration. At the maximum in the solubility, there is a shift between amino acid species controlling the total solubility. At this point, the concentration of the acid species and chloride ion are such that they now equal the solubility limit given by eq 7. Further addition of acid means that the chloride ion concentration is increased, thereby forcing the concentration of the acid form of the amino acid to be reduced. The relative concentrations of the neutral and acidic species are still governed by eq 3 so that a drop in the acid species concentration also reduces the neutral species concentration. K_{sp} is not necessarily a constant at these high acid and solute concentrations, which can possibly account for the sharp drop in solubility after the peak and the apparent increase in the solubility at HCl concentrations greater than 20 wt % HCl.

The solubilities of amino acids in HCl solutions have also been presented as a function of pH. This is shown in Figure 4 for pH values between the isoelectric point (5.94) and 0, corresponding to HCl concentrations between 0 and 8.5 wt %. pH values for higher HCl concentrations were not determined because they were outside the measurement range of the probe (0–14). Therefore, only solubility data up to approximately the peak concentration are presented in Figure 4.

Needham et al. (1971) have presented data for several other amino acids displaying solubility characteristics similar to those shown in Figure 4. In their study, the pH ranged from about 1 to 10, and they found that invariant solubility bands existed over a range of 2–3 pH units on either side of the isoelectric point. This was referred to as the isoelectric band, pH values outside this band resulted in an increase in solubility. Although only pH values below the isoelectric point have been investigated in the present study, the isoelectric band is also apparent for L-Ile. Because Needham et al. worked in the pH range between 1 and 10, they did not report a maximum in solubility as was found in the present work. However, their work did show that the solubility relationships in acidic and basic solutions were approximately symmetrical. Although no supporting data are reported, it is expected that the solubilities of amino acids also reach a maximum upon addition of bases at pH values greater than 10. A complete study of the solid phase in equilibrium with the saturated liquors at various HCl liquor concentrations was not made. Only crystals from solutions with no HCl added or with HCl concentrations greater than 10 wt % were analyzed. Crystals in suspensions without HCl consisted of the anhydrous zwitterion. Crystals in the very acidic slurries were the hydrochloric acid salt with one water of hydration, L-Ile·HCl·H_2O.

Salting Out L-Ile·HCl·H_2O. Equations 6 and 7 indicate that the addition of chloride ion to a saturated solution in which the acid species of an amino acid controls the solubility results in a decrease in solubility. This explained the maximum in L-Ile solubility as liquor HCl concentration was increased and was supported by determining that L-Ile·HCl·H_2O was the solid phase in equilibrium with liquors having HCl concentrations greater than that at which the peak solubility occurred. Experiments in which NaCl, KCl, and NH_4Cl were added to aqueous solutions of L-Ile and HCl were conducted to test this hypothesis. By eq 7, the solubility of L-Ile should be lowered and L-Ile·HCl·H_2O should be precipitated regardless of the source of chloride ion. This is known as the common ion effect on solubility.

In these experiments, the composition of the initial solution to which salt was added was 30 wt % L-Ile and 8.5 wt % HCl; this corresponded approximately to the saturation concentration at the peak solubility. Upon addition of the chloride salts to the solution, there was significant crystallization, indicating a lowering of the solubility of L-Ile. Analyses of liquor and crystal samples from the equilibrated slurries are given by Zumstein (1987). In experiments where all the added salt dissolved, the recovered crystals were composed entirely of L-Ile·HCl·H_2O. Concentrations of up to 30 g of salt/100 g of H_2O were found in equilibrated liquors where the salt was NaCl, KCl, or NH_4Cl. These salts have solubilities in pure water of 36.2 g/100 g of H_2O for NaCl, 35.5 g/100 g of H_2O for KCl, and 39.0 g/100 g of H_2O for NH_4Cl (Mullin, 1971). Therefore, the solubility of the salts was not drastically affected by the L-Ile and HCl in the liquor.

Crystallization of L-Ile·HCl·H_2O upon addition of chloride salts has confirmed that the solubility of L-Ile in the original solution is reduced as expected from eq 7. However, to determine if the solubility is lowered in a manner similar to that caused by the addition of HCl, liquor concentrations from the equilibrated salt slurries were compared with the solubilities in HCl solutions. In order to compare the L-Ile solubilities in the different solutions on a consistent basis, concentrations were expressed in molar quantities. Figure 5 shows the solubility data of L-Ile in the HCl solutions in terms of moles of L-Ile/100 mol of solution and the equilibrium liquor concentrations from the salt addition experiments. Solubilities have been related to the total chloride ion concentration in the liquor

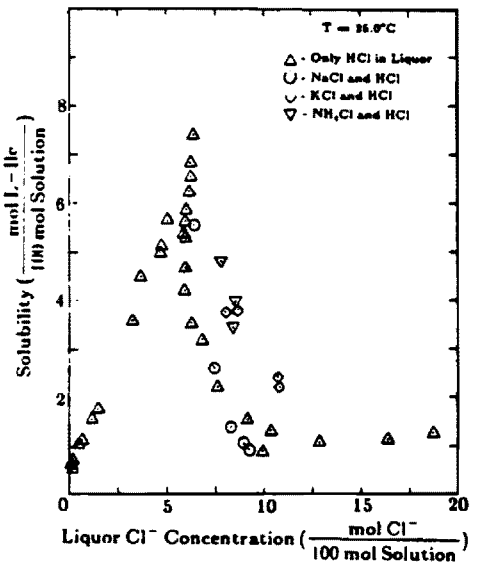

Figure 5. Effect of chloride ion on the solubility of L-isoleucine.

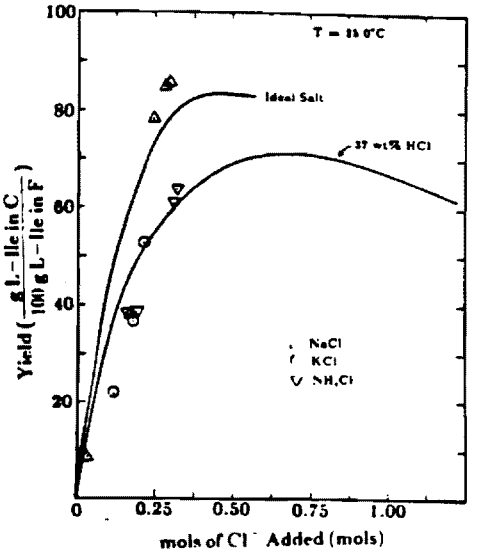

Figure 6. Yield of L-isoleucine from precipitation as Ile-HCl-H_2O using various sources of chloride ion.

from both HCl and chloride salt. NaCl and HCl reduced the solubility of L-Ile in a similar way, but KCl and NH_4Cl did not reduce the L-Ile solubility as much as HCl. These results indicate that, although eq 7 is valid for the effect of chloride ion on L-Ile solubility, the value of the K_{sp} is dependent on the cation accompanying the chloride ion.

Precipitation of the hydrochloric acid salt by addition of HCl is one means of recovering L-Ile from fermentation broths. This is commonly referred to as a salting-out process. The yield of L-Ile from this process, defined as the ratio of the L-Ile in the recovered crystals to the amount of L-Ile in the initial charge, is limited by HCl having to be added as an aqueous solution. Pure HCl is a gas at room temperature, and addition in this state would present special operational problems. Typically, HCl is available as a 37 wt % solution. The yield is limited when HCl is added as diluted solution because H_2O is also added and dissolves more L-Ile. This is shown in Figure 6 for the addition of a 37 wt % HCl solution to an initial charge of 100 g consisting of 30 wt % L-Ile and 8.5 wt % HCl. The theoretical yields were calculated from material balances and based on the solubility data displayed in Figure 2. An outline of these calculations is provided by Zumstein (1987). The maximum yield of L-Ile from addition of 37 wt % HCl to the initial charge is approximately 70% and occurs after about 75 g of 37 wt % HCl solution is added. The addition of a greater amount of acid results in a net dissolution of L-Ile because the solubility of L-Ile is not lowered enough to account for the L-Ile that is dissolved by the added water.

The salt addition experiments in this study showed that the addition of chloride ion salts also causes precipitation of L-Ile-HCl-H_2O from this initial charge. Taking advantage of the common ion effect, chloride ion salts can be added as the precipitant in the process without the addition of water. Since water is not added, chloride salts can increase the yield of the L-Ile from the process. This is shown in Figure 6 for an ideal chloride salt, which causes the reduction in the solubility similar to HCl. Yields of L-Ile are increased to above 80%. However, as shown in Figure 5, the cation of the chloride salt does have some effect on the reduction of the L-Ile solubility. Because KCl and NH_4Cl did not reduce the solubility as much as HCl, the yields for the addition of these salts were less than the ideal salt. However, NaCl reduced the solubility of the L-Ile similar to HCl and did produce yields that were similar to the theoretical estimates.

Conclusions

An improved estimate for the solubility of L-Ile in water is given by eq 1. Deviations from the solubilities determined by Dalton and Schmidt (1935) are thought to be due to unknown impurities in the material used by Dalton and Schmidt. The effect of HCl on the solubility of L-Ile is significant and shown in Figure 2. The strong dependence of the solubility on HCl concentration is due to different L-Ile species controlling the solubility: at low acid concentrations, the total solubility of L-Ile (all forms) is controlled by the zwitterion form of L-Ile; however, the solubility increases as the acid is added to the solution because more of the soluble acidic form of L-Ile is created. At the peak in solubility, the solution reaches a solubility limit for the acid form so that this species now controls the total solubility. Further addition of acid to the solution results in salting out L-Ile-HCl-H_2O. Because of the common ion effect, the addition of chloride salts also caused similar precipitation of L-Ile-HCl-H_2O. However, the reduction in L-Ile solubility due to the addition of chloride ion is dependent on the accompanying cation of the salt. When added to solutions at the solubility peak, NaCl reduces the solubility of L-Ile in a manner similar to the effect of HCl; however, KCl and NH_4Cl do not cause as much of a reduction in solubility as did HCl. Instead of using aqueous solutions of HCl as a precipitant in the recovery of L-Ile from fermentation broths, greater yields can be achieved using NaCl as the precipitant because of the absence of additional water solvent.

Acknowledgment

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Registry No. L-Ile, 73-32-5; L-Ile-HCl, 17694-98-3.

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GENERAL RESEARCH

Development of New Adhesion Agents for Asphalt Cement

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New adhesion and antistripping additives for bitumens were prepared by reacting tetraethylenepentamine (TEPA) with formaldehyde (CH_2O) or with CH_2O and phenol. The additives were characterized both chemically and physically. Adhesion of two bitumens containing 0.2% by weight additive was measured by an improved stripping test and by a U-peeling test. The results showed very good improvement of the antistripping properties of the tested bitumens, especially after water immersion. Both groups of additives (i.e., TEPA–CH_2O and TEPA–CH_2O–phenol) are suggested for use as adhesion agents. During additive formulation, basicity and distribution of polar and basic groups play a major role, together with the capability of the additive to control the bitumen viscosity and consistency.

Adhesion agents are added to asphalt cement to prevent binder-to-aggregate bond separation, especially in wet conditions. If this bond is broken, water will displace the bitumen film over the aggregate surface. The adhesion agents generally used are based on amines, especially fatty polyamines, and amine derivatives such as amides, substituted imidazoline, etc. (Johnson, 1942; Blair et al., 1957; Kalinonski and Crews, 1957; Gianattasio, 1971). Tertiary nitrogen heterocyclic material is proposed to reduce moisture-induced damage in asphalt–aggregate mixtures (Plancher and Petersen, 1982).

The first patents of practical interest appeared in the 1940s. Technical and scientific literature is very poor on this subject, and most additive formulations are, of course, strictly confidential. The effects of antistripping additives on the properties of asphalt cement were studied by Andersen et al. (1982), who also reviewed the literature on the subject, and by Plancher et al. (1982) and Ensley (1973), who studied asphalt–aggregate interactions.

The purpose of this work was to prepare and test adhesion agents whose properties (i.e., basicity, density, viscosity, and adhesion) could be modified as needed, depending on the type of application and bitumen.

Experimental Section

Bitumen and Aggregate Used in the Study. The literature indicates that water stripping resistance is a function of aggregate type and of the asphalt composition (Domaney, 1968; Fromm, 1974). However, for the tests, only one type of aggregate material was used, i.e., the Italian S. Fedelino granite (a silicate rock), because it is representative of the type of material prescribed for the surface layer of asphalt cement in this country.

Table I. Properties of the Bitumens

	Vega	Iranian
penetration (ASTM D5), dm	56	90
softening pt (ASTM D 36), °C	60.0	41.8
penetration index	+1.2	−2.6
Fraass pt (IP 80), °C	−16	−13
asphaltenes (IP 143), wt %	24.6	8.60
acidity (ASTM D 664), mg KOH/g	1.35	0.21

In order to reduce the number of variables, most experiments were carried out with a bitumen obtained from the Sicilian crude Vega; adhesion tests were repeated by using a bitumen obtained from Iranian heavy crude. The characteristics of the bitumens are shown in Table I.

Additive Preparation and Characterization. Various products were prepared by reacting, in different conditions, some polyalkylenepolyamines with other substances; after preliminary adhesion tests, two groups of additives were selected with different molecular structures: (A) tetraethylenepentamine (TEPA)–formaldehyde derivatives prepared by reacting various amounts of CH_2O

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Georgia Institute of Technology

School of Chemical Engineering

QUARTERLY PROGRESS REPORT

(Fall 1989)

presented by,

Hervé CHARMOLUE

Advisor: Dr. R. W. ROUSSEAU

TABLE OF CONTENTS

1- LITERATURE SURVEYS

The purpose of this chapter is to review the work done on inclusion phenomena and habit modification during crystallization. The first part of each section deals with some general background on the topic of interest, while the second part focuses on previous studies and recent developments made in this area. The structure of serine crystals is given at the end of the chapter.

1-1 Literature survey on inclusions

The term "inclusion" denotes any foreign matter-either in a gaseous, liquid or solid form-enclosed within a crystal. The term "occlusion" generally applies to the adhesion of a fluid to the crystal surfaces or to the trapping of solvent between agglomerated crystals. Wilcox (1968) provides extensive reviews of inclusion phenomena. Various terms are used to describe inclusions. These include negative crystals, veils, phantoms, clouds, bubbles ... Generally, a distinction is made between primary inclusions (occurring during crystal growth) and secondary inclusions (occurring after crystal growth).

Primary inclusions. The process begins with a depression in the surface. The depression can result from the presence of a foreign body (either a bubble, a pocket of immiscible liquid or a solid particle) which, by blocking the solution from the crystal, initiates an inclusion. However, the depression can also arise from a variation in supersaturation along the crystal surface. The solution near the edges of a crystal appears to be more supersaturated than at the center because of diffusion along the surface. Therefore, the edges grow faster leading to a depression in the center of the face. As the growth rate is increased, the depth of the depression increases and the growth in the extreme case becomes dentritic. Later on, the cavity can be closed over if the growth rate slows down and the surface becomes increasingly more planar.

Secondary inclusions. The most likely process for the formation of an inclusion after crystal growth is by precipitation. An impurity present in concentrations exceeding its solid solubility, may precipitate giving rise to the simultaneous presence of different phases. Other phenomena which occur after growth do not create new inclusions, but rather modify the shape of existing inclusions. Among these are thermal constraints, minimization of

the surface energy between inclusion and crystal, and coalescence of nearly touching inclusions.

The presence of pockets of solvent trapped as a second phase in crystals is widely reported in the literature (Belyustin and Fridman, 1966, Brooks et al., 1968). It has been recognized that the destabilization of the interface between the crystal face and the supersaturated solution is mainly responsible for inclusion formation. Chernov (1963, 1964) developed a simple criterion for crystal face stability. Denbigh and White (1966) suggested that the interface can only be stable in the presence of a finite interfacial temperature gradient. They pointed out the existence of a critical size for the crystals and a critical growth rate below which inclusion does not form. Reid et al. (1970) observed that the formation of inclusions increases with increasing crystal growth rate and decreases with increasing interfacial temperature gradient. Brice and Burton (1974) showed that interfacial kinetics could produce a stabilizing effect on the interface which would decrease with increasing crystal growth rate. Mullin and Jancic (1979) studied the effects of impurities on stability of the crystal interface. Edie and Kirwan (1973) derived a relationship between process parameters during crystallization and system physical properties to predict solution trapping. A modified form of this relationship was proposed by Myerson and Kirwan (1977). They were able to correlate experimental data using a computer simulation. Hiquily, Laguérie and Coudert (1985) observed the influence of process variables such as cooling rate and agitation rate on the size and moisture due to inclusion of ammonium perchlorate crystals. They also found that a surfactant (sodium dodecyl sulfate) reduced the crystals size and their moisture.

Inclusions are undesirable. They are a source of troubles in crystallization. Physical properties of crystals can drastically be degraded by the presence of inclusions. For example, in micro-electronic applications they cause light scattering, strain defects and semiconductor junctions shorting. They also contribute to caking problems by seepage of the liquid out of stored crystals. The inclusions may even contained more impurities than the mother liquor because of impurities rejection during growth.

Several techniques have been proposed to remove inclusions from crystals. It has been observed that the presence of ionic impurities (Pb^{2+}, Ni^{2+}...) could prevent the formation of inclusions. Ultrasonic vibrations during crystallization have been tried but with limited success. Heating near of the boiling point of an included solvent often failed to remove it from the crystal. Moreover, heating

causes cracking or complete breakage of the crystal. Even heating to decrepitation does not guarantee the destruction of all the inclusions. An alternative solution is the use of a temperature gradient to move the inclusions through the crystals (Wilcox, 1968). Since the solubility is temperature dependent, crystalline material dissolves on the high-solubility side of the inclusion and crystallizes out on the low-solubility side. This technique is only feasible for large crystals.

1-2 Literature survey on habit modification

The habit of a crystal is defined as its external shape, resulting from different rates of growth of the faces (Mullin, 1972). Even though belonging to the same crystal system (tetragonal, orthorhombric or hexagonal for example), crystals of a given substance obtained under various conditions may exhibit completely different appearance. A crystallization method may favor the formation of needles (acicular habit) while another may give a tabular habit (plates). In any cases, the habit is the consequence of the relative growth rates of the faces of the crystals: the smaller the growth rate, the more extensive the development of the face. This is schematically represented in Figure 1-1. The faces of lowest growth rates develop at the expense of the faces of greatest growth rates.

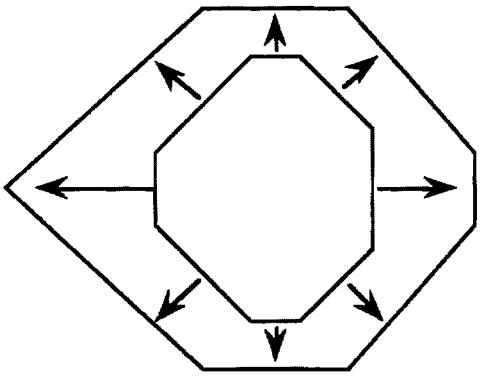


Figure 1-1: Schematic representation of a habit change

The habit of a crystal can be affected by numerous factors. Theorically, any agent affecting the surface activity of a crystal could be listed as one of these factors. The degree of supersaturation, the pH of the solution, the type of solvent, the presence of impurities, the rate of agitation, the temperature of crystallization are some examples among these factors. Mullin

(1972) has listed the major parameters affecting crystal habit. There are in the literature numerous references to habit modification, but many of these are qualitative. Boistelle and Astier (1988) have studied the effect of supersaturation on the habit of barbey ß-amylase crystals. Black and Davey (1988) explained the habit changes caused by a number of amino acids on crystals of L-asparagine in terms of surface structure. Michaels and Colville (1960) used face growth rate measurements to predict crystal habit. Zumstein and Rousseau (1989) observed the influence of the type and amount of surfactant on habit and size of L-isoleucine crystals. Addadi et al. (1982) have pioneered a structural approach to habit modification of organic crystals.

In spite of the availability of extensive experimental data, the mechanism of crystal habit modification has not been established. There are many views on the nature of this mechanism. Some investigators believe that considerable changes in the composition and properties of the solution surrounding the crystal are responsible for habit modification. Another group of investigators attributes habit modification to surfactant effect, which alters the surface energy of crystal faces. A third group of researchers assumes that an impurity alters the crystal habit when its structure is related in a certain way with the structure of the crystallizing substance. It has also been suggested that the influence of impurities on the crystal habit is due to their selective adsorption on crystal faces.

In conclusion, we may say that the crystal habit depends on internal factors (such as structure and bonds) and on external factors (such as crystallization conditions and solution composition). In all the cases, there must be a change in the surface activity of the crystal faces for an habit modification to occur. This change may result from chemical bonding or physical adsorption.

1-3 The crystal structure of serine

The crystal structure of DL-serine has been determined by Albrecht et al. (1943) and Shoemaker et al. (1953). They found that the crystal lattice belongs to the monoclinic space group. The unit cell contains four molecules of serine and has the following values for the lattice constants:

a_0=10.72 Å, b_0=9.14 Å, c_0=4.825 Å, ß=106°27'

The general configuration of a molecule with interatomic distances is indicated in Figure 1-2. The molecular distribution within the crystal is illustrated in Figure 1-3. As is usual in amino acid crystals, the molecules are tied together through a system of hydrogen bonds. Each amino nitrogen has three of these, two to carboxyl oxygen and one to hydroxyl oxygen. The N-O (carboxyl) hydrogen bonds link the molecules together into sheets parallel to {010} (or b-face); the N-O (hydroxyl) hydrogen bond can be thought of as tying these sheets to one another. The positions for the hydrogen atoms, including those involved in these bonds, have been computed on the assumptions that C-H = 1.09Å, N-H = 1.01Å, O-H = 0.97Å, and that the angles of their bonds are close to tetrahedral.

One of the main characteristics of serine is the relatively large number of different types of H-bonds which can be found. Recently, Schroetter et al. (1985) have postulated the possible existence of C-H...O hydrogen bonds in the solid state. Masamura (1987, 1988) used a computational procedure to determine the optimized structure of serine. His conclusion is in agreement with the early work of Shoemaker (1953). Van Alsenoy et al. (1988) performed a conformational analysis of serine. They were able to design fourteen conformations with significantly different features but similar energies. They concluded that H-bonding was an important factor determining conformational stability in serine. Simultaneous H-bonding between different groups in the molecule may form bi-, tri-, and tetra-cyclic systems.

The crystallographic data reported here refer to DL-serine crystals, that is a solid mixture of the D- and L-forms. These two forms are stereoisomers. Due to the presence of an asymmetric carbon in the serine molecule, it is possible to design two non superimposable configurations symmetric about a plane (like an object and its image in a mirror). The two configurations exhibit similar chemical and physical properties except optical properties. The presence of only one form in the crystal should impose new geometric constraints and as a result the crystal structure of L-serine may differ considerably from those of DL-serine. Unfortunately, I did not find in the literature any crystallographic data on L-serine. However, the information on the hydrogen bonds linking the molecules together may be helpful in understanding an habit modification.

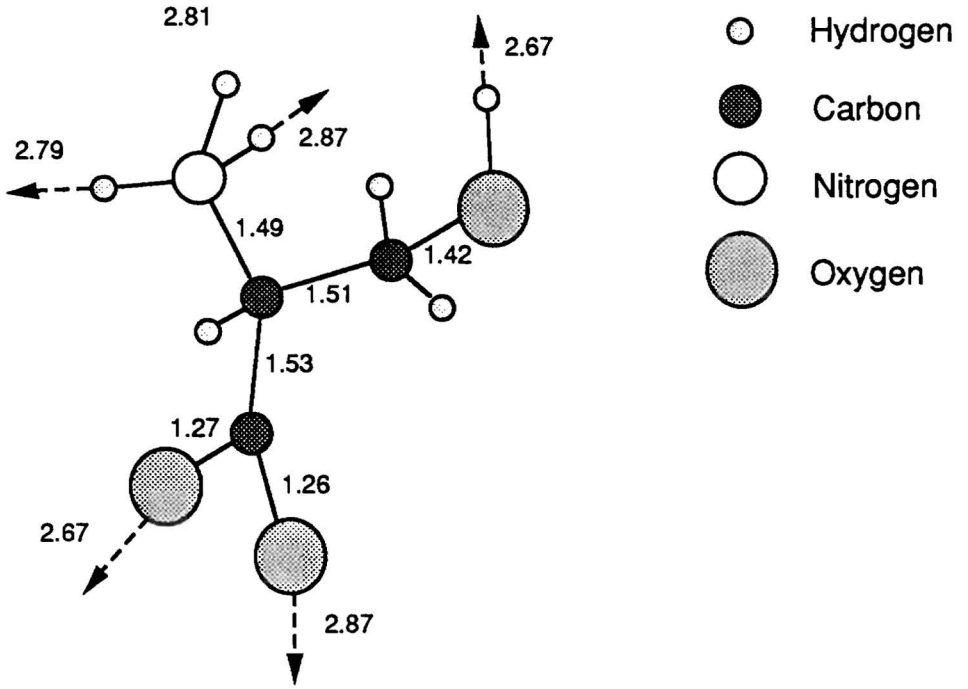


Figure 1-2: Bond dimensions in DL-serine crystals. Dashed lines indicate the intermolecular hydrogen bonds. (Shoemaker, 1953).

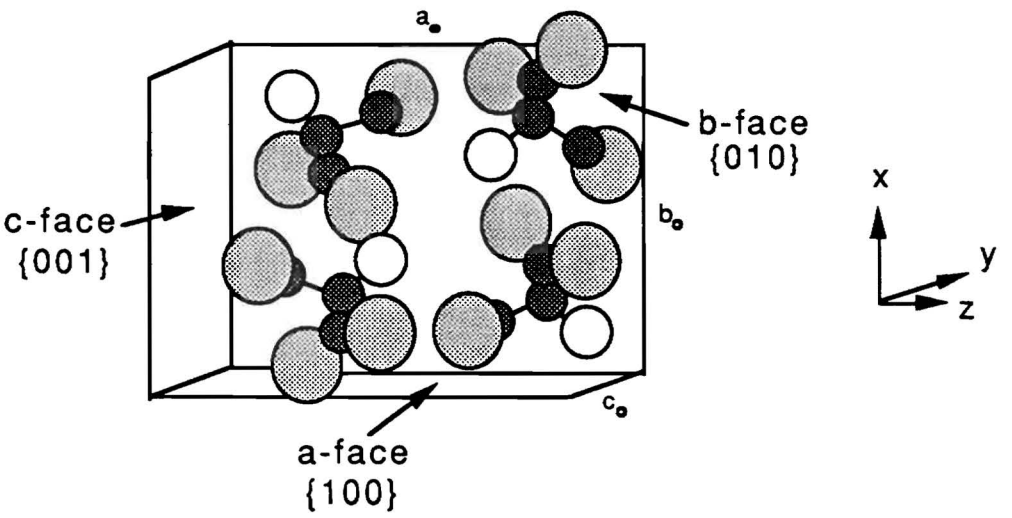


Figure 1-3: The monoclinic structure of the unit cell of DL-serine. (Shoemaker, 1953).

2- SOLUBILITY OF L-SERINE IN WATER/METHANOL SOLUTIONS

2-1 Experimental procedure

Distilled water was used in all experiments. ACS-grade methanol was purchased from Fisher Scientific. L-serine was provided by Ajinomoto Inc. and was used without any further purification.

A known volume of methanol was mixed to a known volume of water in a 20 ml vial. An excess of L-serine crystals was added, then the vial was sealed with a stopper and placed in a water bath. The temperature was controlled by a Precision Circulating System to ±0.05°C of the setpoint and was measured with a calibrated thermometer. The slurry was heated 10°C above the desired temperature for an hour, cooled to the desired temperature and allowed to equilibriate. Twelve hours later, samples were removed and filtered through a 0.45 μm glass fiber filter. The amount of L-serine in the filtrate was determined by HPLC analysis. The pH of the solution was at the isoelectric point (pH=5.68).

Table 2-1: Solubility of L-serine in water/methanol mixtures at 10°C and 30°C.

Methanol vol. %	L-ser g/100ml T=10°C	L-ser g/100ml T=30°C
0	22.715	39.404
10	18.902	33.039
10	19.055	33.897
20	11.576	24.380
20	11.592	24.276
30	5.951	16.900
30	6.879	16.874
40	3.957	9.259
40	4.453	8.643
60	1.637	2.748
60	1.970	2.493
80	0.838	0.962
80	0.864	0.969
100	0.592	0.701
100	0.708	0.705

2-2 Results and discussion

The solubility data of L-serine in water/methanol mixtures were obtained over the range 0 to 100% methanol (vol. %) at 10°C and 30°C. The data are summarized in Table 2-1 and shown in Figure 2-1.

According to the pH of the solution, amino acids can be present within a solution in acidic, basic or zwitterionic form (Figure 2.2).If the pH is less then the isoelectric point, the following equilibrium is established between the acidic (A^+) and zwitterionic ($A^\pm$) forms:

$$A^\pm + H_3O^+ \rightleftharpoons A^+ + H_2O \qquad \text{(equ. 2-1)}$$

$$K_1 = \frac{(A^\pm)\,(H_3O^+)}{(A^+)} \qquad \text{(equ. 2-2)}$$

$$pH = pK_1 + \log\frac{(A^\pm)}{(A^+)} \qquad \text{(equ. 2-4)}$$

If the pH is greater than the isoelectric point, the basic (A^-) and zwitterionic forms are in equilibrium as follows:

$$A^\pm + H_2O \rightleftharpoons A^- + H_3O^+ \qquad \text{(equ. 2-6)}$$

$$K_2 = \frac{(A^-)\,(H_3O^+)}{(A^\pm)} \qquad \text{(equ. 2-7)}$$

$$pH = pK_2 + \log\frac{(A^-)}{(A^\pm)} \qquad \text{(equ. 2-8)}$$

K_1 and K_2 are the equilibrium constants. pK_1 and pK_2 are the ionization constants for the acidic and basic forms respectively. Equations 2-4 and 2-8 are known as the Henderson-Hasselbalch equations. They relate the relative concentrations of the species in solution with the pH. Utilizing equations 2-4 and 2-8, the magnitudes of the acidic, basic and zwitterionic forms with respect to the pH of the solution have been calculated. The ratio of existence of the different species is shown in Figure 2-3.

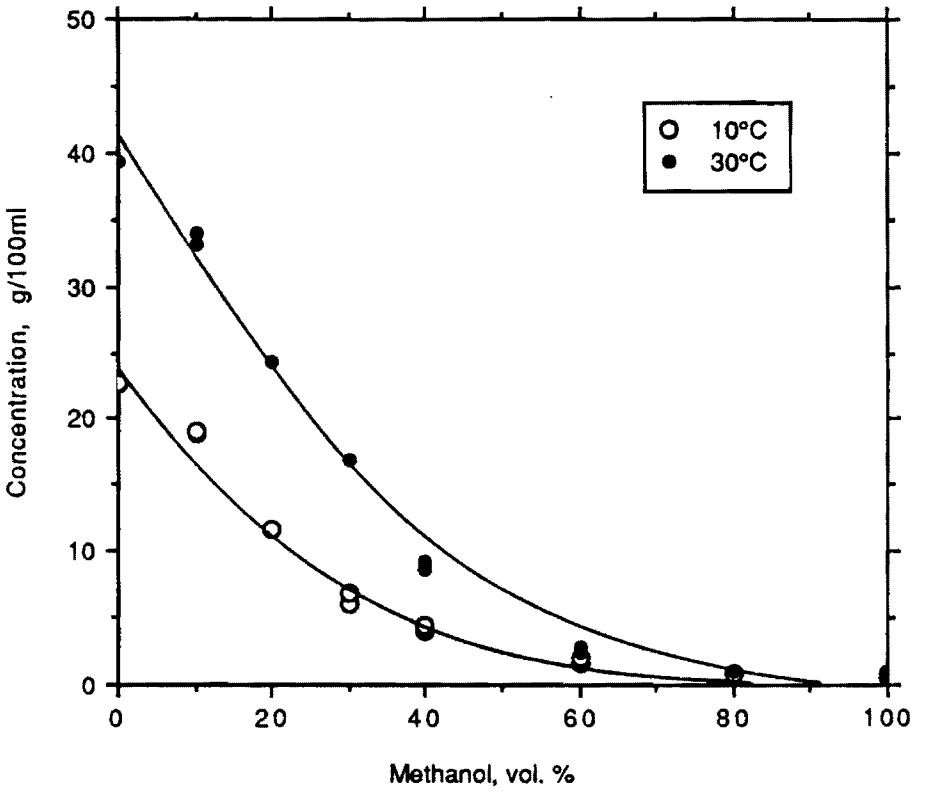


Figure 2-1: Solubility of L-serine in mixtures of water and methanol.

During the solubility measurements, the pH of the solution was near the isoelectric point. As a result, L-serine was present in the zwitterionic form (figure 2-3). The molecule has no net charge. However, it behaves as a strong dipole in solution. The amino and carboxyl groups are hydrophilic in character. They constitute the polar "head" of the amino acids. On the other hand, the side chain of an amino acid can be either hydrophobic or hydrophilic. L-serine belongs to the last category. This can explain its relative high solubility in water. The solubility is temperature and pH dependant. It is also strongly influenced by the nature of the solvent. When methanol is switched from water, the solubility of L-serine drastically changes as shown in Figure 2-1. Let us define the relative solubility of L-serine by the ratio of its solubility in a mixed solvent system to its solubility in water at the same temperature. Figure 2-4 shows the variations of the relative solubility with the composition of the mixed solvent.

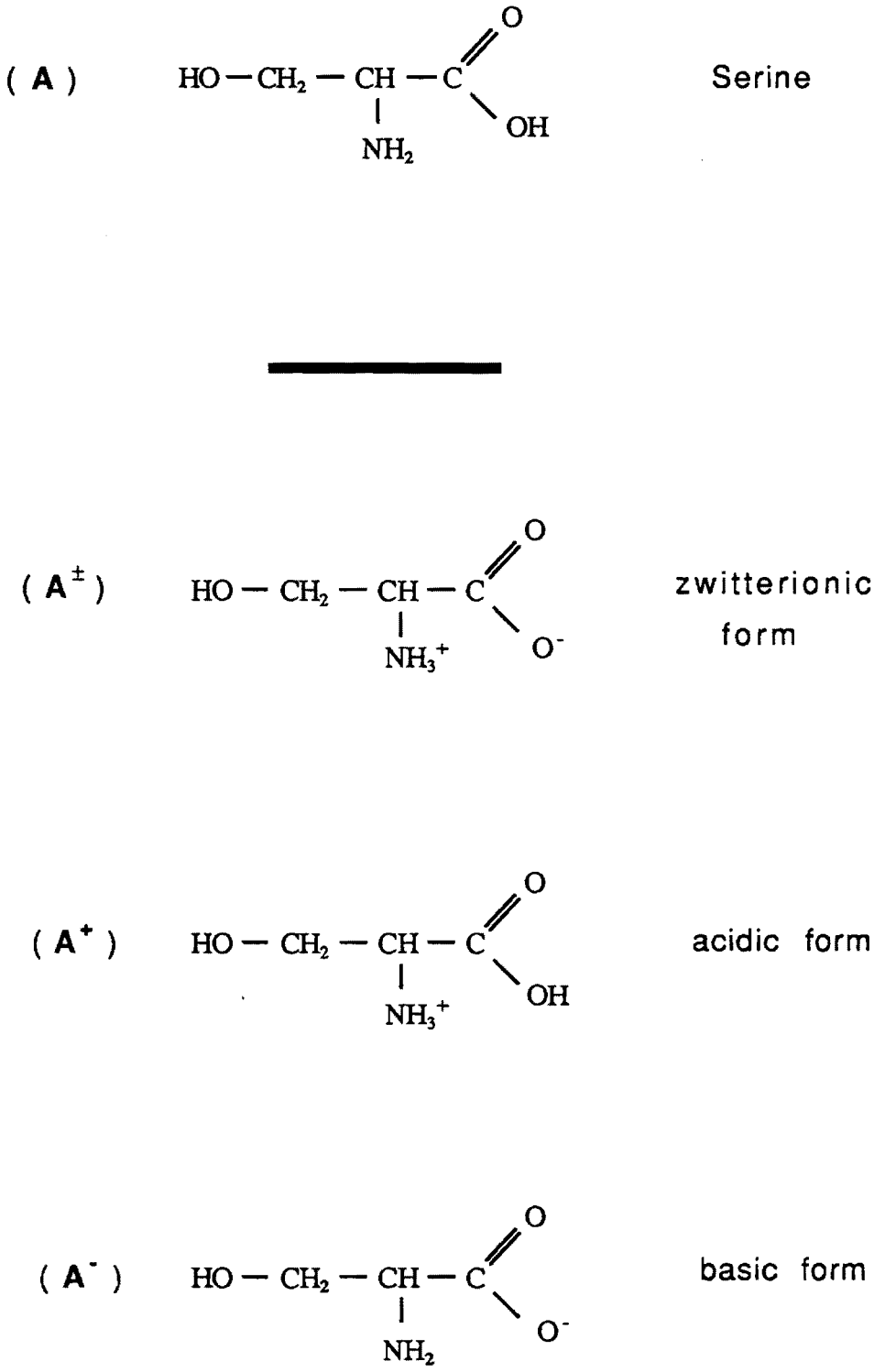


Figure 2-2: Acidic, basic and zwitterionic forms of serine.

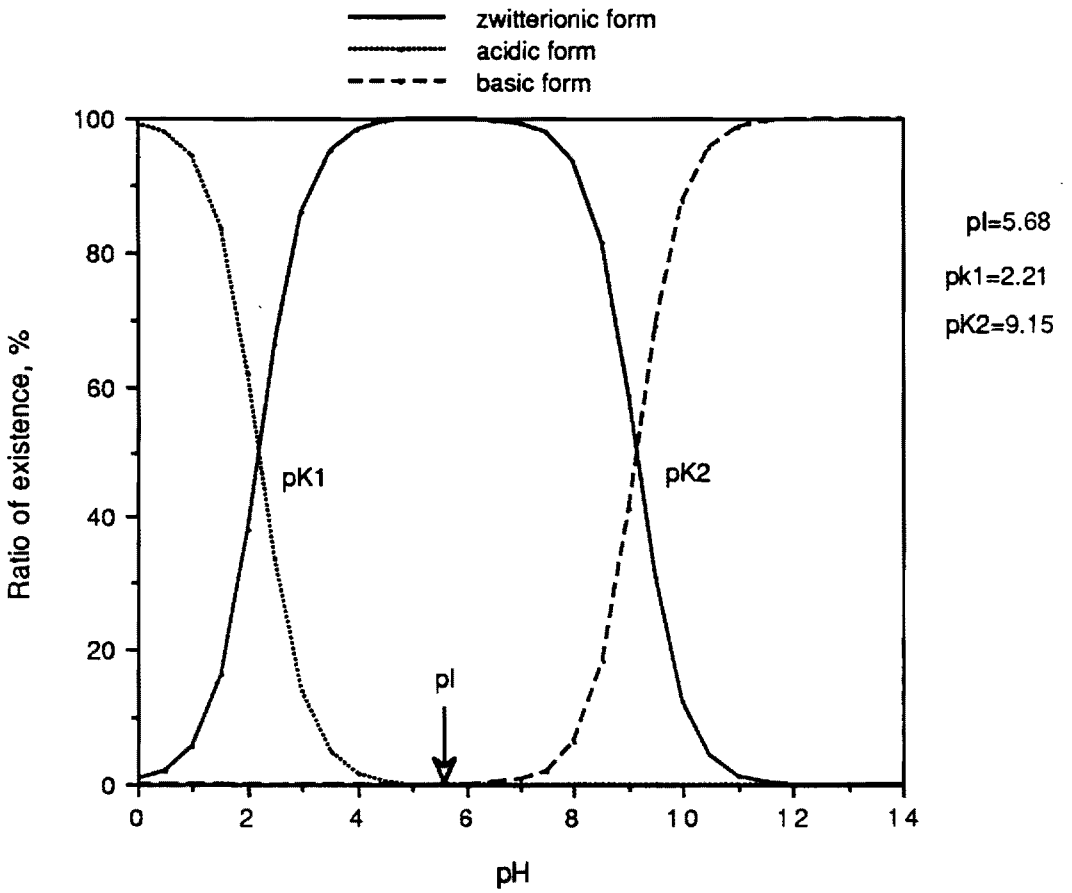


Figure 2-3: Dissociation curve of L-serine.
(T=25°C)

Orella and Kirwan (1989) have studied the effects of different aliphatic alcohols on the solubilities of several amino acids. They observed that the magnitude of the change is smaller for amino acids with a non-polar side chain than for those with a polar side chain. Therefore, alcohols would be better crystallizing agents in terms of yield for amino acids with polar side chains. The data for serine is an illustration. The relative solubility is reduced by two orders of magnitude. The reduction factor seems to be the same at 10 and 30°C. The molecular interactions between the solvent and the polar groups of L-serine lead to a change in the solubility. This change can be explained in terms of solvent composition dependance of the activity coefficient of the solute. The estimation of solubility change depends on the ability to predict activity coefficient. Unfortunately, there is so far no recommended expression to correlate activity coefficients of amino acids. The difficulty resides in the complex behavior of these systems in solution. They exhibit

chemical properties similar to those of weak electrolytes (dissociation in acidic, basic or zwitterionic forms), combined with different types of interactions with the surrounding (ionic, polar, dipolar or non-polar). An attempt in deriving solid-liquid equilibria expression suitable for amino acids has been made by Nass (1988). The activity coefficient is separated into two independent terms, one arising from chemical interactions and the second from physical interactions. The chemical term is modeled by chemical theory while the physical interactions are accounted for by a form of the Wilson equation. Orella and Kirwan (1989) provide a review of the methods that can be employed in order to correlate the activity coefficients.

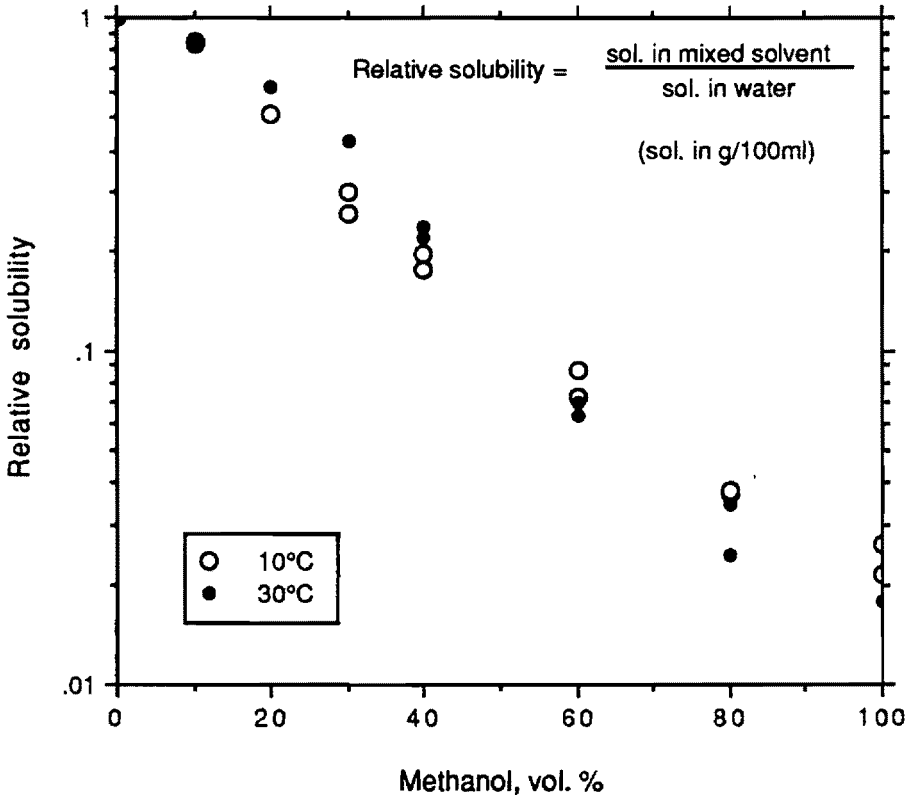


Figure 2-4: Relative solubility of L-serine.

3- GAS CHROMATOGRAPHY ANALYSIS

The objective of this section is to develop an experimental procedure to analyze the residual amount of methanol in L-serine crystals. Gas chromatography (GC) has established itself as a classical method for methanol analysis. However, two major problems have to be overcome in our case. On one hand, very dilute solutions have to be used to minimize the problems associated with L-serine crystals inside the column of the GC. On the other hand, the detection method has to be very sensitive in order to detect at such low levels. After having demonstrated that a thermoconductivity type detector was not appropriate, we decided to use a flame ionization detector (FID).

3-1 Experimental procedure

The analyses were conducted on a Varian-3700 gas chromatograph with flame ionization detector. The separation was accomplished using a 6' x 1/4" glass column packed with Porapak Q, 80/100. The following settings have been retained. They insure reasonable analysis time, good resolution and sensitivity.

Injection volume: 0.5µl
Temperature:
- injector: 220°C
- column: 160°C
- detector: 230°C

Flowrate:
- carrier gas: 40 ml/min (Helium)
- air: 240 ml/min
- hydrogen: 60 ml/min

To avoid discrepancies in injection volume, an internal standard has been used in the sample preparation. Ethanol was chosen as internal standard. 100 µl of a 1% vol. ethanol solution were mixed to 10 ml of the solution to be analyzed. 0.5 µl of the resulting mixture were injected for GC analysis.

3-2 Typical chromatograms and calibration

A typical chromatogram is shown in Figure 3-1. The first peak to show up is air. The second and third peak are due to the methanol and to the internal standard (ethanol) respectively. Below the

chromatogram appear the results of the integration. The response factor can be deduced:

$$\text{Response Factor} = \frac{\text{methanol area}}{\text{int. standard area}}$$

Standard solutions of known methanol concentration were analyzed and the response factor of each was calculated. The values of the different concentrations and their corresponding response factors are reported in Table 3-1 and plotted in Figure 3-2. The concentrations are expressed in µg of methanol per ml of solution.

Table 3-1: Response Factor-Concentration conversion data for methanol.

Concentration µg/ml	Response Factor
1980.0	22.157
1584.0	17.493
990.0	11.091
792.0	8.890
396.0	4.395
79.2	1.080
39.6	0.646

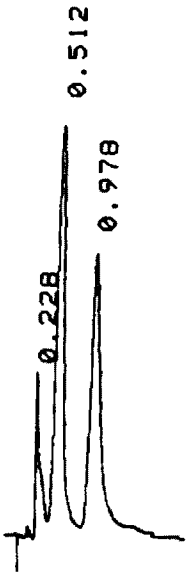
According to Figure 3-2, the response factor and the concentration are linearly related in the domain 0-2000 µg/ml. A linear regression of the data gives the following results:

$$\mathbf{R.\ F.} = a_1 + b_1\,\mathbf{C} \qquad \text{(equ. 3-1)}$$

where

R. F. = response factor
C = concentration in µg/ml
a_1 = 0.10492
b_1 = 0.01107

The correlation coefficient is 1. The fit of the data is shown in Figure 3-2.



PEAK NO	PEAK NAME	RESULT	TIME (MIN)	AREA COUNTS	SEP CODE
1		14.0659	0.227	32265	BV
2		10.0770	0.517	23115	VB
3		75.8571	0.973	174004	BB
TOTALS:		100.0000		229384	

MULTIPLIER: 1.00000

Figure 3-1: Typical chromatogram from GC analysis (79.2 µg/ml of MeOH).

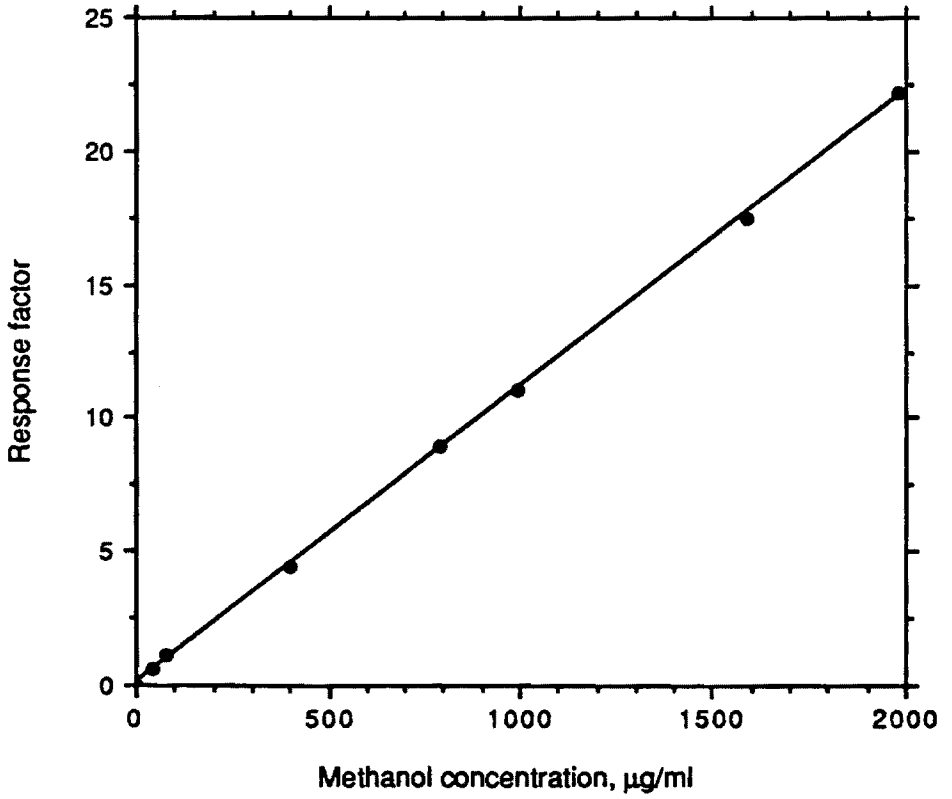
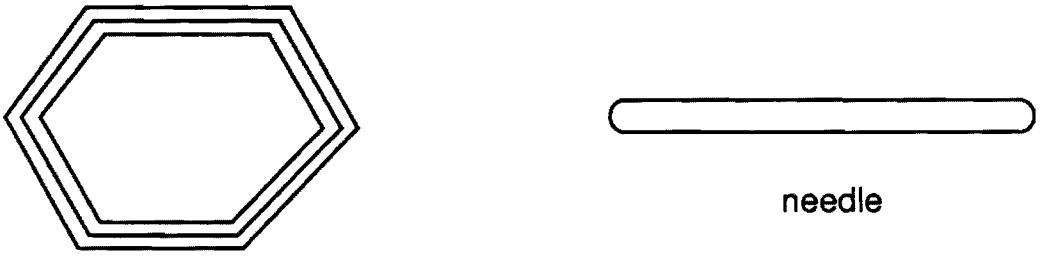


Figure 3-2: Response factor-concentration conversion curve for methanol.

4- HABIT OF L-SERINE CRYSTALS

Interesting aspects related to crystals morphology of amino acids have been outlined by Zumstein and Rousseau (1989). Observations of L-serine crystals produced by different methods reveals that the conditions of crystallization affect strongly the shape of the crystals. Figure 4-1 shows the habit modification when two different crystallization techniques are used. Crystallization by cooling favors the formation of hexagonal crystals, while the use of methanol as precipitant gives a needle-like habit.



hexagonal

Crystallization from aqueous solutions

Crystallization from methanol/water mixtures

Figure 4-1: Habit change of L-serine crystals.

To be able to explain this habit modification in terms of crystal surface structure, crystallographic data on L-serine are required. As mentioned before, only information concerning DL-serine was found in the literature. Therefore, we decided to observe the behavior of DL-serine crystals in the hope of identifying the factors which would be responsible for the habit modification.

4-1 Case of DL-serine

DL-serine crystals were produced by cooling a supersaturated solution (7g of DL-serine in 100ml) from 50 to 10°C. They exhibit the same thin hexagonal blade shape as L-serine crystals obtained under the same conditions. Methanol was then used as precipitant agent in crystallizing DL-serine from aqueous solutions. Surprisingly, no modification of the crystal habit was noticed. This seems to indicate that in fact L-serine and DL-serine crystals differ considerably in structure. Also, the solubilities of the L- and DL-

forms are totally different and this is in accordance with the previous conclusion.

4-2 Discussion

In absence of data on the structure of L-serine crystals, our understanding of the mechanism of habit modification is quite speculative. We can draw the following conclusion: the relative growth of the faces of the crystal is strongly affected by the presence of methanol in the solution. We have demonstrated that in fact the hydroxyl group is responsible for the habit change. Ethylene glycol ($HO\text{-}CH_2\text{-}CH_2\text{-}OH$) induced the same modification of the morphology of the crystals. Two possible assumptions may be put forward, the OH-group may favor the growth of certain faces, and/or it may inhibit the growth of the others. The interaction of the hydroxyl group with a specific face may be electrostatic. A hydrogen bond would form with a nitrogen atom (because of the similarity between MeOH and the hydroxyl branch of the L-serine (Figure 4-2), and thus by blocking L-serine molecules from the crystals surface would prevent the face to grow.

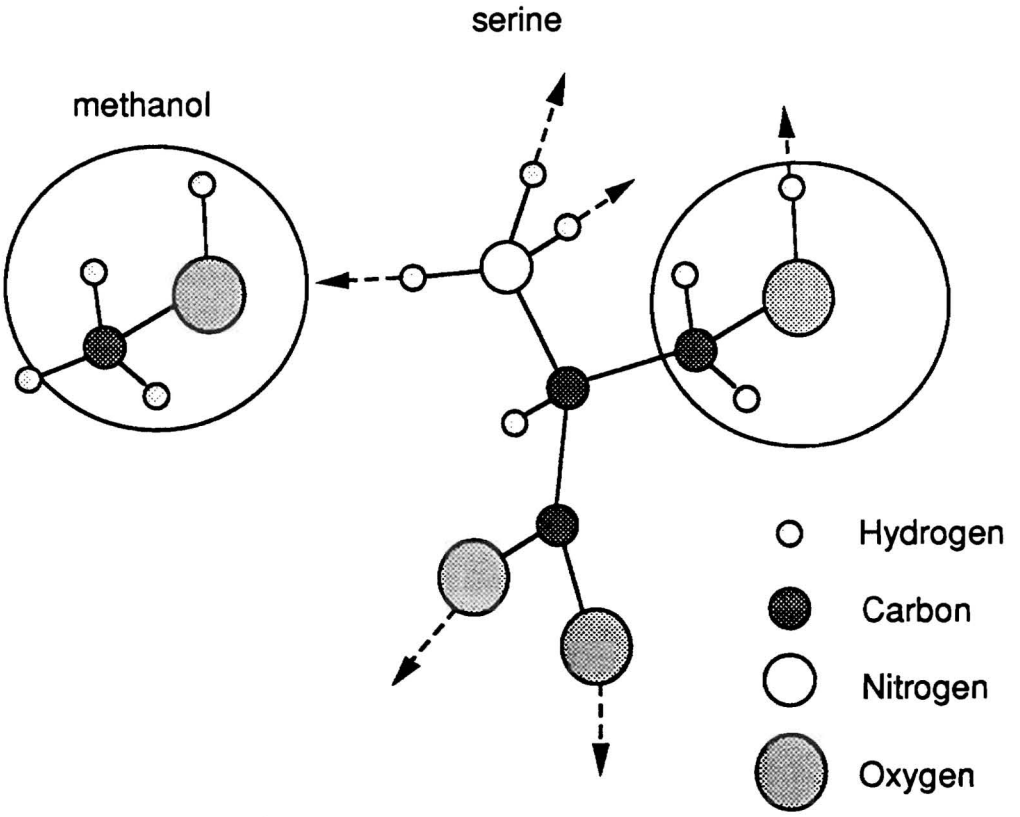


Figure 4-2: Similarity between methanol and hydroxyl group of L-serine.

5- FUTURE WORK

In the upcoming quarter, the projected work is as follows:

- Crystallization of L-serine at different process variables
  - Influence of the cooling rate
  - Influence of the agitation rate
  - Influence of the methanol addition rate
  - Modification of the purification process (cooling and methanol addition will be considered as two distinct operations in the purification process, then these two operations will be combined)

- Characterization of the resulting crystals:
  - HPLC for determination of L-serine and impurities concentration
  - GC analysis for residual amount of methanol
  - Microscopic observation and photographs to determine the habit and the size of the crystals

- Solubility measurements of DL-serine in water.

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NATIONAL SCIENCE FOUNDATION
FINAL PROJECT REPORT

PART I - PROJECT IDENTIFICATION INFORMATION

1. Program Official/Org. NAME UNAVAILABLE; ADDRESS TO A/D FOR CTS

2. Program Name INTERFACIAL, TRANS & SEPARATION PROCESS

3. Award Dates (MM/YY) From: 01/88 To: 06/92

4. Institution and Address
GA Tech Res Corp - GIT
Administration Building
Atlanta GA 30332

5. Award Number 8722281

6. Project Title
Crystal Purity Habit and Size Distribution from Batch Crystallization

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And 1 Return Envelope

NSF Grant Conditions (Article 17, GC-1, and Article 9, FDP-II) require submission of a Final Project Report (NSF Form 98A) to the NSF program officer no later than 90 days after the expiration of the award. Final Project Reports for expired awards must be received before new awards can be made (NSF Grant Policy Manual Section 677).

Below, or on a separate page attached to this form, provide a summary of the completed project and technical information. Be sure to include your name and award number on each separate page. See below for more instructions.

PART II - SUMMARY OF COMPLETED PROJECT (for public use)

The summary (about 200 words) must be self-contained and intelligible to a scientifically literate reader. Without restating the project title, it should begin with a topic sentence stating the project's major thesis. The summary should include, if pertinent to the project being described, the following items:

- **The primary objectives and scope of the project**
- **The techniques or approaches used only to the degree necessary for comprehension**
- **The findings and implications stated as concisely and informatively as possible**

PART III - TECHNICAL INFORMATION (for program management use)

List references to publications resulting from this award and briefly describe primary data, samples, physical collections, inventions, software, etc. created or gathered in the course of the research and, if appropriate, how they are being made available to the research community. Provide the NSF Invention Disclosure number for any invention.

	12-29-92
Principal Investigator/Project Director Signature	**Date**

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Part II – SUMMARY OF COMPLETED PROJECT

There is little understanding of the important factors influencing the use of batch crystallization for purification of solutes from complex mixtures. It is recognized generally that separation of resultant crystals from liquor is affected strongly by the nature of the crystalline product but there is less awareness that other properties (e.g. purity, appearance, drug efficacy, tableting characteristics, etc.) are also influenced by crystal properties.

The systems chosen for study in the completed research were amino acids having importance in their own right. Two types of purification problems were addressed: (1) those arising from mixtures containing impurities having molecular structures similar to the primary solute and (2) precipitants added to induce crystallization. The experiments were conducted in small glass vessels whose temperature was carefully controlled and to which either acid or base was be added at an appropriate rate and suitable conditions. Careful and precise analytical measurements determining solution and crystal compositions were essential, and the key instrument was the HPLC.

L-Isoleucine is synthesized commercially by fermentation and is used in intravenous feeding solutions. Recovery with a good yield and high purity is essential. Possible contaminants in the fermentation broth include other amino acids such as valine, leucine, and alpha amino butyric acid. Recovery and purification of isoleucine by crystallization requires precipitation of the acid form by adding HCl to a batch crystallizer containing a solution of the neutral material and controlled concentrations of the potential amino acid contaminants mentioned above. In this research the effects of crystallization protocol properties affecting the final product are to be determined. The issues resolved through the completed research include the following:

- Solubilities of isoleucine, leucine, valine in neutral, acidic and basic solutions were determined and shown to depend on temperature, pH, and concentration of various other compounds.
- Factors affecting the purity of recovered isoleucine crystals were quantified. These included solution composition, mixing, and rate of precipitant addition.
- The effects of different acids and/or bases (precipitating agents) on the purity of recovered isoleucine crystals were determined.

L-serine also is synthesized by fermentation and its recovery involves several crystallization steps, one of which is precipitation with a non-solvent. The non-solvent of choice has been methanol but this substance has been found to be incorporated in the recovered crystals. As there are stringent limitations on allowable methanol content, the supported research was aimed at determining those factors that control inclusion of solvent in the crystals. Factors resolved include the effect of methanol on crystal habit; determination of the mechanism by

which methanol incorporates in the crystal; the effect of crystal washing on methanol content; and the influence of operating variables (rate of precipitant addition, rate of cooling, and mixing) on the methanol content of the recovered crystals.

The results of the sponsored research will improve the processes involved with recovery and purification of a number of pharmaceutical compounds, especially those used as model systems. Improvements will reduce the cost of the operations and facilitate maintaining the quality of product materials from one batch to the next. Finally, the results of this work will be useful in troubleshooting those processes with which difficulties have been encountered in obtaining desired crystal purities, morphologies, or sizes.

PART III – TECHNICAL INFORMATION

Presentations Resulting from Research

"Use of Crystallization for Recovery and Purification of L-Isoleucine and Other Biologically Produced Materials," Department of Chemical Engineering, Louisiana State University, Baton Rouge, LA, March 1988.

"Crystallization for Separation and Purification," Dow Chemical Company, Midland, MI, March 1988.

"Relating Crystal Properties to Crystallizer Operation," Ajinomoto USA, Raleigh, NC, September 1988.

R. W. Rousseau, "Removal of Trace Impurities by Crystallization," AIChE Annual Meeting, Washington, DC, November 1988.

"Crystallization Processes for Recovery and Purification of High-Value Chemicals," Department of Chemical Engineering, Auburn University, Auburn, AL, January 1989.

"Crystallization Processes for Recovery and Purification of High-Value Chemicals," Department of Chemical Engineering, University of New Mexico, Albuquerque, NM, January 1989.

"Crystallization Processes for Recovery and Purification of High-Value Chemicals," Department of Chemical Engineering, Texas A&M University, College Station, TX, February 1989.

"Use of Crystallization for Separation and Purification, Or.....What's New in Crystallization," Separations Symposium, E. I. DuPont, Wilmington, DE, April 1989.

"Separation and Purification of Amino Acids by Crystallization," Engineering Foundation Conferences, Davos, Switzerland, May 1989.

"Principles of Batch Crystallization," General Electric Specialty Chemicals, Morgantown, WV, July 1989.

"Yield and Purity of L-Isoleucine Recovered by Crystallization," Ajinomoto Co., Inc., Technology and Engineering Center, Central Research Laboratories, Kawasaki, Japan, August 1989.

"Separation and Purification by Crystallization," Department of Chemical Engineering, Purdue University, West Lafayette, IN, October 1989.

T. Gambrel and R. W. Rousseau, "The Effects of Crystallizer Operating Variables on Crystal Purity," AIChE Annual Meeting, San Francisco, CA, November 1989.

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"Crystallization for Separation and Purification," General Electric Corporate Research and Development Center, Schenectady, NY, February 1990.

"Separation and Purification of Specialty Chemicals by Crystallization," Exxon Research and Engineering, Clinton, NJ, April 1990.

"Separation and Purification of Specialty Chemicals by Crystallization," Department of Chemical Engineering, University of Massachusetts, Amherst, MA, April 1990.

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H. Charmolue and R. W. Rousseau, "Factors Affecting the Purity of L-Serine Crystals," 11th Symposium on Industrial Crystallization, Garmisch-Partenkirchen, West Germany, September 1990.

"Purification of Specialty Chemicals by Crystallization," Department of Chemical Engineering, West Virginia University, Morgantown, WV, October 1990.

"Separation and Purification: Needs and Opportunities for Crystallization Research," Oktoberfest Symposium in Honor of Drs. James R. Fair, John J. McKetta, and Howard F. Rase, Department of Chemical Engineering, University of Texas, Austin, TX, October 1990.

"Use of Crystallization for the Separation and Purification of Specialty Chemicals," Royal Institute of Technology, Stockholm, Sweden, November 1990.

H. Charmolue and R. W. Rousseau, "Effects of Methanol on the Solubility and Habit of L-Serine," Annual AIChE Meeting, Chicago, IL, November 1990.

"Affecting the Purity, Habit, and Size of Amino Acid Crystals," California Institute of Technology, Pasadena, CA, January 1991.

"Concerns Related to Crystal Quality," NSF Workshop on Opportunities and Challenges in Crystallization Research, Iowa State University, Ames, IA, March 1991.

"Purification of Amino Acids by Crystallization," Department of Chemical Engineering, Princeton University, Princeton, NJ, April 1991.

"Recovery of Biological Products by Crystallization," Fourth World Congress of Chemical Engineering, Karlruhe, Germany, June 1991.

"Crystallization/Precipitation in Separations Involving Dilute Solutions," NSF Workshop on Separations from Dilute Solutions," University of Cincinnati, Cincinnati, OH, June 1991.

"Purity, Habit and Size Distribution of Amino Acid Crystals," Engineering Foundation Conference on Separation Technology, Kona, HI, October 1991.

"Controlling Morphology and Purity in Crystallization Processes," DuPont Chemicals Creativity Committee, Wilmington, DE, February 1992.

"Factors Affecting Crystal Morphology," Second Annual Meeting of the Association of Crsytal Technology, Kingsport, TN, February 1992.

"Purification of Specialty Chemicals by Crystallization," Sydney Chapters of the Institution of Engineers Australia and the Institution of Chemical Engineers (London), Sydney, Australia, September 1992.

"Crystal Morphology: Opportunities for Modifying Crystal Shape and/or Polymorphic Structure," Department of Chemical Engineering, University of Newcastle, Newcastle, Australia, September 1992.

"Purification of Specialty Chemicals by Crystallization," Newcastle Chapter of the Institution of Engineers Australia, Newcastle, Australia, September 1992.

"Affecting Crystal Purity, Morphology, and Size Distributions," Queensland Chapters of Intitution of Engineers Australia and IChemE(London), Brisbane, Australia, September 1992.

"Purification of Specialty Chemicals," Department of Chemical Engineering, University of Queensland,Brisbane, Australia, September 1992.

"Affecting Crystal Purity, Morphology, and Size Distributions," Western Australia Chapter of the Instituion of Engineers Australia, Perth, Australia, September 1992.

"Factors Affecting Crystal Purity, Morphology, and Size Distributions," South Australian Chapter of the Institution of Engineers Australia, Adelaide, Australia, September 1992.

"Purification of Specialty Chemicals by Crystallization," Department of Chemical Engineering, Melbourne University, Melbourne, Australia, September 1992.

"Crystallization Processes and Research," Melbourne Chapter of the Institution of Engineers Australia, Melbourne, Australia, September 1992.

S. Furuta, R. W. Rousseau and A. S. Teja, "Production of Amino Acid Fine Particles using Rapid Expansion of Subcritical Fluid Solutions," Annual AIChE Meeting, Miami Beach, FL, November 1992.

H. E. Canossa and R. W. Rousseau, "Effect of L-Leucine and L-Valine on the Purity, Size, and Morphology of L-Isoleucine Crystals," Annual AIChE Meeting, Miami Beach, FL, November 1992.

Publications Resulting from Research

R. C. Zumstein and R. W. Rousseau, "Solubility of L-Isoleucine in and Recovery from Neutral and Acidic Aqueous Solutions," *Industrial & Engineering Chemistry Research*, **28**, 1226–1231(1989).

R. C. Zumstein, T. Gambrel and R. W. Rousseau, "Factors Affecting the Purity of L-Isoleucine Recovered by Batch Crystallization," *ACS Symposium Series 438, Crystallization as a Separations Process*, A. S. Myerson and K. Toyokura, eds., 85–99, 1990.

Hervè Charmolue and R. W. Rousseau, "L-Serine Obtained by Methanol Addition in Batch Crystallization," *AIChE Journal*, **37**, 1121–1128(1991)

H. Charmolue and R. W. Rousseau, "Factors Controlling the Purity of L-Serine Crystals," *Proceedings of the 11th Symposium on Industrial Crystallization*, A. Mersmann, ed., pp. 379–384, 1990.

R. W. Rousseau, "Concerns Related to Crystal Quality," *Proceedings of the Workshop on Opportunities and Challenges in Crystallization Research*, 69–84, 1991.

M. Gatewood Brown and R. W. Rousseau, ``pH and Maximum Solubilities of L-Isoleucine, L- Leucine, and L-Valine," submitted for publication.

H. Canossa Koolman and R. W. Rousseau, "Effect of L-Leucine and L-Valine on the Purity, Size, and Morphology of L-Isoleucine Crystals," in prepararation.

Theses Resulting from Research

Hervè Charmolue, "The Effects of Process Variables on Purity, Size and Habit of L-Serine Crystals Recovered by Batch Crystallization," M.S. Thesis, 1990

Timothy Gambrel, "Batch Crystallization and Crystal Purity," M.S. Thesis, 1989.

Marena Gatewood, "Solubility and Recovery of L-Isoleucine from High pH Solutions and the Cause of L-Serine Habit Differences When Crystallized from Water and Methanol-Water Solutions," M. S. Thesis, 1992.

Hannia Canoosa Koolman, "Effect of Impurities and Agitation on Purity, Size, and Morphology of L-Isoleucine Crystals Obtained from Batch Crystallizers," M.S. Thesis, 1992.

PART IV — FINAL PROJECT REPORT — SUMMARY DATA ON PROJECT PERSONNEL

(To be submitted to cognizant Program Officer upon completion of project)

The data requested below are important for the development of a statistical profile on the personnel supported by Federal grants. The information on this part is solicited in response to Public Law 99-383 and 42 USC 1885C. All information provided will be treated as confidential and will be safeguarded in accordance with the provisions of the Privacy Act of 1974. You should submit a single copy of this part with each final project report. However, submission of the requested information is not mandatory and is not a precondition of future award(s). Check the "Decline to Provide Information" box below if you do not wish to provide the information.

Please enter the numbers of individuals supported under this grant.
Do not enter information for individuals working less than 40 hours in any calendar year.

	Senior Staff		Post-Doctorals		Graduate Students		Under-Graduates		Other Participants[1]	
	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.	Male	Fem.
A. Total, U.S. Citizens	1	0	0	0	2	1	0	1	0	0
B. Total, Permanent Residents	0	0	0	0	0	1	0	0	0	0
U.S. Citizens or Permanent Residents[2]:										
American Indian or Alaskan Native										
Asian								1		
Black, Not of Hispanic Origin					1	1				
Hispanic						1				
Pacific Islander										
White, Not of Hispanic Origin					1					
C. Total, Other Non-U.S. Citizens										
Specify Country										
1. France					1					
2.										
3.										
D. Total, All participants (A + B + C)	1	0	0	0	3	2	0	1	0	0
Disabled[3]										

☐ Decline to Provide Information: Check box if you do not wish to provide this information (you are still required to return this page along with Parts I-III).

[1]Category includes, for example, college and precollege teachers, conference and workshop participants.

[2]Use the category that best describes the ethnic/racial status for all U.S. Citizens and Non-citizens with Permanent Residency. *(If more than one category applies, use the one category that most closely reflects the person's recognition in the community.)*

[3]A person having a physical or mental impairment that substantially limits one or more major life activities; who has a record of such impairment; or who is regarded as having such impairment. *(Disabled individuals also should be counted under the appropriate ethnic/racial group unless they are classified as "Other Non-U.S. Citizens.")*

AMERICAN INDIAN OR ALASKAN NATIVE: A person having origins in any of the original peoples of North America, and who maintain cultural identification through tribal affiliation or community recognition.

ASIAN: A person having origins in any of the original peoples of East Asia, Southeast Asia and the Indian subcontinent. This area includes, for example, China, India, Indonesia, Japan, Korea and Vietnam.

BLACK, NOT OF HISPANIC ORIGIN: A person having origins in any of the black racial groups of Africa.

HISPANIC: A person of Mexican, Puerto Rican, Cuban, Central or South American or other Spanish culture or origin, regardless of race.

PACIFIC ISLANDER: A person having origins in any of the original peoples of Hawaii; the U.S. Pacific Territories of Guam, American Samoa, or the Northern Marianas; the U.S. Trust Territory of Palau; the islands of Micronesia or Melanesia; or the Philippines.

WHITE, NOT OF HISPANIC ORIGIN: A person having origins in any of the original peoples of Europe, North Africa, or the Middle East.